Morphometric Analyses of Ears in Two Families of Pinnipeds

By

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Abstract

Pinniped (seal and sea lion) auditory systems operate in two acoustically distinct environments, air and water. Pinniped species differ in how much time they typically spend in water. They therefore offer an exceptional opportunity to investigate aquatic versus terrestrial hearing mechanisms. The Otariidae (sea lions and fur seals) generally divide their time evenly between land and water and have several adaptations; *e.g.* external pinnae, related to this lifestyle. Phocidae (true seals) spend the majority of their time in water; they lack external pinnae and have well developed ear canal valves.

Differences in hearing ranges and sensitivities have been reported recently for members of both of these families [Kastak, D., Schusterman, R.J., 1998. Low frequency amphibious hearing in pinnipeds. J. Acoust. Soc. Am. 1303, 2216-2228.; Moore, P.W.B., Schusterman, R.J., 1987. Audiometric assessment of northern fur seals, *Callorhinus ursinus*. Mar. Mamm. Sci. 3, 31-53.]. In this project, the ear anatomy of three species of pinnipeds: an otariid, the California sea lion (*Zalophus californianus*), and two phocids, the northern elephant seal (*Mirounga angustirostris*) and the harbor seal (*Phoca vitulina*), was examined using computerized tomography (CT scans) and gross dissection. Three-dimensional reconstructions of the heads and ears from CT data were used to determine interaural dimensions and ossicular chain morphometrics. Ossicular weights and densities were measured conventionally. Results strongly support a canal-centric system for pinniped sound reception and localization. Further, true seals show adaptations for aquatic high frequency specialization.

Key Words: Middle ear; Binaural hearing; Pinnipeds; Marine mammals; Seals;

1. Introduction

Thirty-four species of pinnipeds (eighteen true seals, ten fur seals, five sea lions and one walrus) inhabit the earth today (King 1983). These mammals divide their time between land and water; breathing air yet spending much of their lives underwater. Seals and sea lions belonging to two closely related families (phocids and otariids) which arose from a common terrestrial carnivore (Bininda-Emonds *et al.* 1999; Flynn and Nedbal 1998; Ledje and Arnason 1996a, 1996b; Lento *et al.* 1995; Wyss 1987) approximately 25 million years ago (Barnes *et al.* 1985). When the ancestor of modern seals and sea lions started venturing into water, its ears began the transition to an amphibious structure. However, despite their common ancestry, each pinniped group differs in how much time is spent in water or in air. Consequently, these pinniped families offer an excellent opportunity to investigate the continuum of aerial vs. aquatic adaptations for hearing in mammals.

Pinnipeds utilize many of their senses both in air and underwater, including hearing, vision and tactile (Renouf 1991). In both phocids (true seals) and otariids (sea lions and fur seals), vocalizations are associated with social interactions including territorial behavior, mating, and pup recognition (Thomson and Richardson 1995). Passive localization using acoustic cues is hypothesized to play an important role in numerous activities including foraging, predator avoidance, and navigation (Richardson 1995, Schusterman *et al.* 2000).

Pinnipeds are protected under the United States Marine Mammal Protection Act. This act is designed to protect marine mammals from human interference. It prohibits pinnipeds from being hunted. The law is designed also to defend

marine mammals from harmful anthropogenic influences such as chemical and noise pollution. The more scientists know about species sensitivities, the better basis for governmental regulations that implement this act. For example, knowledge of a species hearing abilities allows the government to set reasonable limits on sound sources used in the ocean that will avoid or minimize impacts.

As a group, little is known about pinniped hearing capabilities. This thesis investigates correlations between pinniped head and ear anatomy and hearing abilities. By examining the relationship between anatomy and behavior, the thesis will generate relationships useful for estimating hearing abilities from anatomy in the 25 untested pinniped species. In addition, these relationships will provide independent verification for hearing curves generated by individual animals; lending confidence to behavioral hearing ability measures often based on a single subject.

1.1 The history of pinniped hearing research

Early published accounts of the pinniped auditory system focused on descriptive anatomy (Rosenthal 1825, Hyrtl 1845, Doran 1878, Zuckerkandl 1896, Denker 1899, Tandler 1899). Thirty years ago, the publication of the first seal behavioral audiogram (Møhl 1968a) changed the emphasis from anatomy to behavior for studying these systems. Additional audiograms followed representing both aerial and underwater thresholds of numerous seal and sea lion species including the harp seal, *Phoca groenlandicus*, (Terhune and Ronald 1971, 1972), the ringed seal, *Phoca hispida*, (Ronald and Terhune 1975), the California sea lion, *Zalophus californianus*, (Schusterman *et al.* 1972; Schusterman 1974), the northern fur seal, *Callorhinus ursinus*, (Moore and Schusterman 1987),

the Hawaiian monk seal, *Monachus schauinslandi*, (Thomas *et al.* 1990), the Pacific walrus, *Odobenus rosmarus*, (Kastelein *et al.* 1996), and the northern elephant seal, *Mirounga angustirostris*, (Kastak and Schusterman 1997). Recent papers (Kastak and Schusterman 1998; Kastak and Schusterman 1999; Kastak *et al.* 1999) have used behavioral techniques to show significant hearing differences between largely aquatic seals (phocids) and largely terrestrial sea lions (otariids). During the last 30 years, some research appeared also on how anatomical and physiological differences among pinnipeds relate to differences seen among the behavioral audiograms. Early work in this area was invasive, including acute investigations of cortical and midbrain responses from implanted electrodes (Bullock *et al.* 1971, Ridgway and Joyce 1975) and cochlear microphonics (Møhl and Ronald 1975, Lipatov 1992). This work was limited to very few species. Consequently, at this time, our knowledge of the anatomy underlying hearing differences among pinnipeds remains limited. This thesis addresses the anatomical issues of air- versus water-adapted ears by utilizing several techniques novel to this field, including tomography (CT scan imaging) and broad species comparisons.

1.2 Mammalian Hearing

Scientists have divided the mammalian ear into three parts: the outer ear, the middle ear and the inner ear (Fig 1). The outer ear functions as a sound collector. In most terrestrial mammals this portion of the ear is an external pinna. Its shape and location play important roles in amplifying certain frequencies of sound while attenuating others. In addition, the positioning of the sound reception point in the head, commonly the pinna, is critical for localization. The

outer ear also includes the external auditory canal, which funnels sound waves to the tympanic membrane. This membrane designates the transition point to the middle ear.

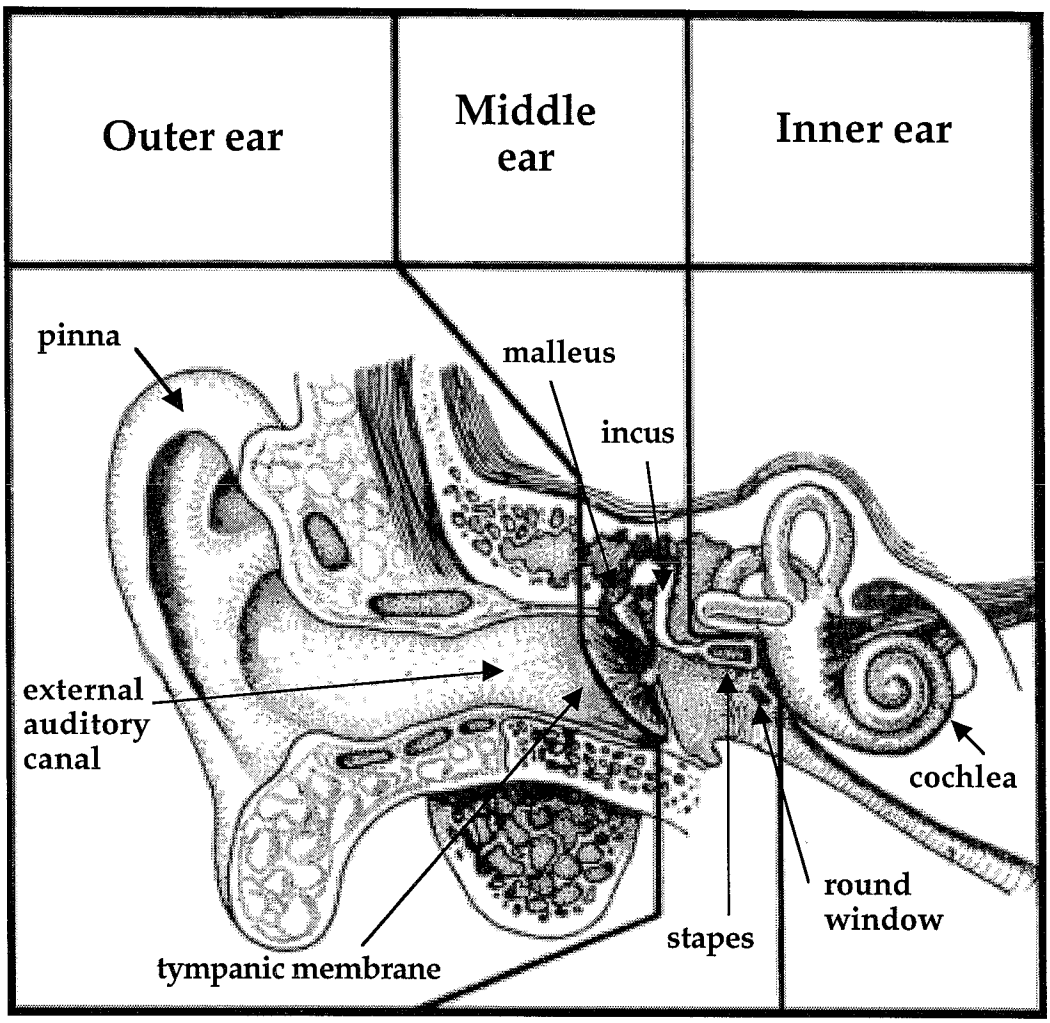


Figure 1. Schematic diagram of the human ear (adapted from Yost 1994).

The middle ear transduces airborne sound waves into mechanical movements. When sound waves reach the tympanic membrane they cause it to vibrate. These vibrations are transmitted to the ossicular chain, which consists of three

bones, the malleus, incus and stapes. The malleus is connected directly to the tympanic membrane. It conducts energy to the incus, which connects to the stapes. The stapes vibrates the oval window membrane, which is the boundary with the inner ear.

Movement of the oval window membrane transmits vibrations representing acoustic energy to the fluid filled cochlea. Vibrations in the cochlear fluid cause the basilar membrane inside the cochlea to undulate. The portion of the basilar membrane which experiences the largest motion depends upon the input frequencies. These movements of the membrane bend cilia on hair cells connected to the membrane, that trigger the release of electrical signals sent by the auditory nerve to the brain. Final information processing steps takes place in the central auditory nervous system.

The description above (see Yost 1994 for further details) illustrates the most common sequence of events in mammalian hearing. However, there are alternative pathways for sound reception in addition to the canal route described above including bone condition and air space conduction. In bone conduction, sound is transmitted directly to the cochlea through skeletal and cranial bones, bypassing the outer and middle ears. In air space conduction, sound energy is transmitted to the cochlea through direct vibration of air spaces in the middle ear rather than the ossicular chain (Yost 1994). The mechanisms pinnipeds use are a matter of contention (Møhl 1968b, Ramprashad *et al.* 1972, Repenning 1972, Terhune 1974, Lipatov 1992, Renouf 1991). Understanding and clarifying the sound conduction paths used in pinnipeds is a major goal of this thesis.

1.3 Comparative studies

Comparative studies are often useful for illuminating broad evolutionary patterns. This study continues this comparative trend, investigating the anatomy behind the hearing differences in pinnipeds by combining tomography (CT scans) with traditional dissection. This pairing of digital with gross dissection gives unique insight into the pinniped auditory system.

Ears from three species of pinnipeds, divided among the Otariidae and Phocidae families, were included in this study. Otariids (sea lions and fur seals) are adapted to a nearly equi-amphibious lifestyle, spending approximately half their time in water and half on land. They are more streamlined than most terrestrial mammals and have reduced pinnae, but this group is agile on land (King 1983). Phocids, however, spend very little time on land. They are even more streamlined and have no external pinnae. This family swims gracefully but moves awkwardly on land. For this study, the otariids were represented by the California sea lion (*Zalophus californianus*); the phocids or true seals were represented by the northern elephant seal (*Mirounga angustirostris*) and the harbor seal (*Phoca vitulina*).

These species were good candidates for anatomical work for two reasons. There is a relatively large body of behavioral work on their hearing abilities (Møhl 1968a, 1968b; Schusterman *et al.* 1972; Schusterman 1974; Moore and Schusterman 1987; Kastak and Schusterman 1998, 1999; Kastak *et al.* 1999; Southall *et al.* 2000). In addition, specimens for dissection are not generally difficult to obtain.

This thesis compares seal and sea lion outer and middle ear anatomy and hearing ability, particularly in the context of pinnipeds as amphibious mammals within larger mammalian data sets. One section focuses on correlations of interaural distances with upper frequency hearing limits (Heffner and Heffner 1992; Ketten 2000). This component addresses the question of which hearing mechanisms are functionally important in pinnipeds. Another section focuses on the correlation of a function of ossicular weight with upper frequency hearing limit. This component concentrates on verifying the functional anatomical basis for middle ear transduction from earlier work by Hemilä *et al.* (1995). A final section examines correlations of upper functional hearing limit with ossicular density. This component also expands on techniques and data from previous studies (Parnell and Dreher 1963, Lees et al 2001).

2. Materials and Methods

2.1 Specimens

This study focused on three species from two families. Live animal measurements were obtained through the New England Aquarium, the Woods Hole Aquarium and SeaWorld of Florida. Specimens for dissection were obtained through the California Marine Mammal Stranding Network, courtesy of National Marine Fisheries Service permit F/SWR3:JGC. Study animals included eight live and thirteen postmortem California sea lions (*Zalophus californianus*), nine postmortem northern elephant seals (*Mirounga angustirostris*), and six live and twenty postmortem harbor seals (*Phoca vitulina*). In general, a broad range of sex-age class combinations was represented in the data set. It was not possible to obtain an adult male northern elephant seal for dissection; therefore in comparison to the other specimens studied only a limited set of measurements from a museum collection skull were obtained for males of this species. Tables 1, 2 and 3 summarize the data for the specimens examined in this study, including age class, sex, and condition.

2.2 External Measures

In vivo measures of external features were taken from animals at the New England Aquarium (Guthrie, PV 18-Lana, Chacoda, Reggae, Rigel) and the Woods Hole Aquarium (PV15-Sandy, PV16-Coco). Parallel measures on postmortem specimens were obtained prior to dissection. Intermeatal distance was measured in two ways: as the straight line distance between the meatus and

Table 1
California sea lion (*Zalophus californianus*) specimens

ID	Age class	Sex	Condition	Place of origin	Procedure/ Measures
ZaC11	NA	NA	Skull	HMCZ	CT
ZaC19	Adult	F	Head	MMC	EM,CT,D,O
ZaC21	Adult	F	Head	MMC	EM,CT,D,O
ZaC22	Adult	F	Head	MMC	EM,CT,D,O
SW1	Adult	M	Live	SeaWorld	EM
SW2	Adult	M	Live	SeaWorld	EM
SW3	Adult	M	Live	SeaWorld	EM
SW4	Adult	M	Live	SeaWorld	EM
SW5	Adult	M	Live	SeaWorld	EM
SW6	Adult	M	Live	SeaWorld	EM
SW7	Adult	M	Live	SeaWorld	EM
SW8	Adult	M	Live	SeaWorld	EM
Guthrie	Adult	M	Live	NEAQ	EM
ZaC23	Adult	M	Head	MMC	EM,CT,D,O
ZaC12	Juvenile	M	Head	MMC	EM,CT,D,O
ZaC15	Yearling	M	Head	MMC	EM,CT,D,O
ZaC01	Yearling	F	Right ear	MMC	O
ZaC02	Yearling	M	Right ear	MMC	O
ZaClit 01	NA	NA	Information	Møhl 1968b	OW
ZaC17	Subadult	M	Head	MMC	EM,CT,D,O
ZaC18	Subadult	M	Head	MMC	EM,CT,D,O
ZaC20	Subadult	M	Head	MMC	EM,CT,D,O

Notes: CT-CT scanned, for internal measures; D-dissection; EM-external measures; F-female; HMCZ-Harvard Museum of Comparative Zoology; M-male; MMC-The Marine Mammal Center, Sausalito, CA; NA-not available; NEAQ-New England Aquarium, Boston, MA; O-ossicles; OW-ossicular weight; SeaWorld-SeaWorld of Florida;

Table 2
Northern elephant seal (*Mirounga angustirostris*) Specimens

ID	Age class	Sex	Condition	Place of origin	Procedure/ Measures
MA21	Adult	M	Head	HMCZ	CT
MA11	Juvenile	F	Head	MMC	EM,CT,D
MA07	Juvenile	NA	Ears	MMC	O
MA14	Yearling	NA	Head	SeaWorld	O
MA13	NA	NA	Head	SeaWorld	EM,CT,D,O
MA23	Adult	F	Head	MMC	EM,CT,D,O
MA24	Juvenile	F	Head	MMC	EM
MA22	Weaner	F	Head	MMC	EM
MA00	NA	NA	Head	MMC	EM, CT,

Notes: CT-CT scanned, for internal measures; D-dissection; EM-external measures; F-female; HMCZ-Harvard Museum of Comparative Zoology; M-male;
MMC--The Marine Mammal Center, Sausalito, CA; NA-Not available; O-ossicles;

Table 3
Harbor seal (*Phoca vitulina*) specimens

ID	Age class	Sex	Condition	Place of origin	Procedure/ Measures
PV18-Lana	Adult	F	Live	NEAQ	EM
Chacoda	Adult	M	Live	NEAQ	EM
Reggae	Adult	M	Live	NEAQ	EM
Rigel	Adult	M	Live	NEAQ	EM
PV14	Juvenile	F	Head	NEAQ	CT,EM
PV15-Sandy	Juvenile	F	Live	WHAQ	EM
PV16-Coco	Juvenile	F	Live	WHAQ	EM
DO6979	Juvenile	M	Whole	NEFSC	EM
DO5476	Juvenile	M	Whole	NEFSC	EM
DO5226	Juvenile	M	Whole	NEFSC	EM
PV10	Juvenile	NA	Head	SeaWorld	CT,EM,D
DO4233	Yearling	F	Whole	NEFSC	EM
DO6976	Yearling	M	Whole	NEFSC	EM
DO5227	Yearling	M	Whole	NEFSC	EM
DO5773	Yearling	M	Whole	NEFSC	EM
DO6977	Yearling	M	Whole	NEFSC	EM
DO6978	Yearling	M	Whole	NEFSC	EM
PV17	Yearling	NA	Head	NEAQ	CT,EM
PVG	Yearling	NA	Whole	NEAQ	CT
PVJ	Yearling	NA	Whole	NEAQ	CT
PV23	Yearling	NA	NA	NEAQ	CT
PV13	Pup	NA	Head	NEAQ	CT
PV22	Pup	NA	Flensed head	NEAQ	CT
Pvlit01	NA	NA	Information	Nummela 1995	OW
Pvlit02	NA	NA	Information	Møhl 1968b	OW
PV06	NA	NA	Flensed head	SeaWorld	CT,O

Notes: CT-CT scanned, for internal measures; D-dissection; EM-external measures; F-female; M-male; NEAQ-New England Aquarium, Boston, MA; NA-not available; NEFSC- Northeast Fisheries Science Center Marine Mammal Archive; OW--ossicular weight; WHAQ-Woods Hole Aquarium, Woods Hole MA;

as the curved distance (shortest external path) over the head between the meatuses (Fig 2). In sea lions, these measurements were taken based on the meatal opening at the base of the pinnae. Other measurements were taken opportunistically. These included head girth, diameter, tip of snout to meatus or base of pinnae, and pinnal or meatal dimensions.

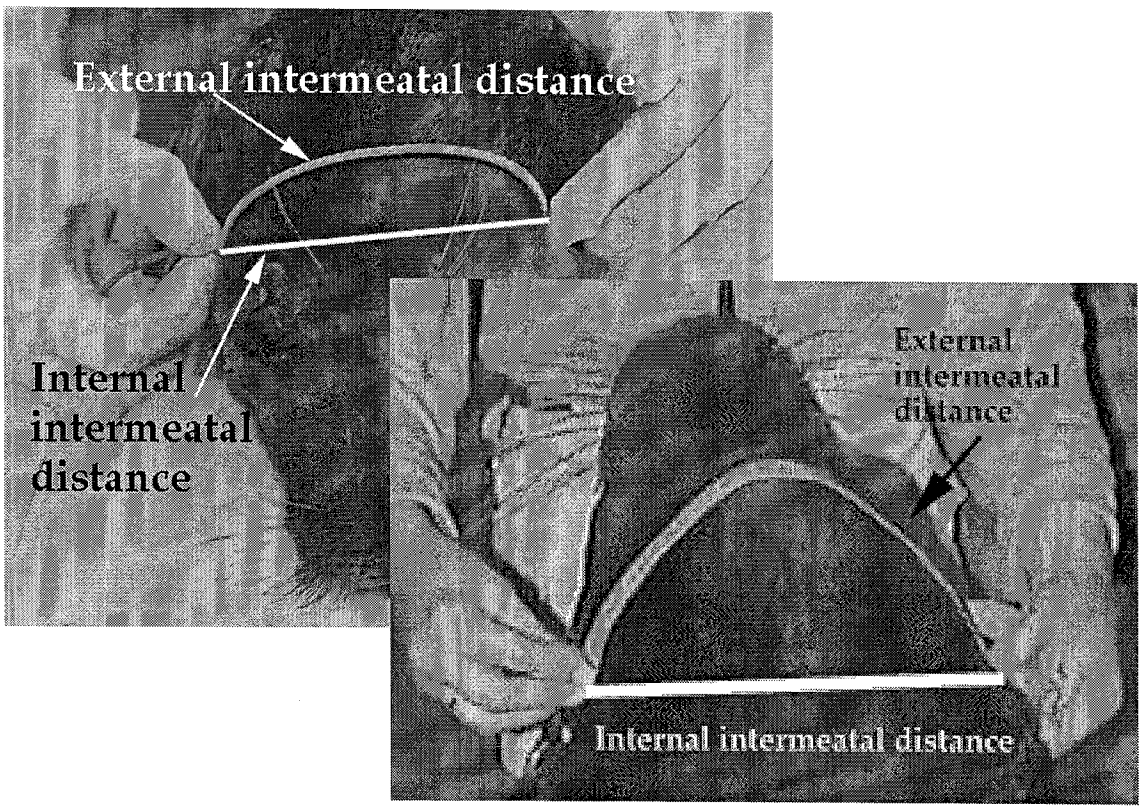


Figure 2 . Intermeatal distances measured on a harbor seal (left) and a California sea lion (right).

2.3 Dissection

All dissections were photo-documented using an Olympus D-600L digital camera. Heads obtained post-mortem were decapitated just behind the occipital condyles at the atlanto-occipital joint. Musculature was dissected away from the external auditory canals. The exposed canals were then measured for straight-line and curvilinear length from meatus or meatal opening at the base of the pinnae to the tympanic membrane. Auditory canal samples were obtained and preserved in ten percent formalin. Musculature and connective tissue were removed from the bullae using scalpels and forceps, and proximal and distal interbullar distances were measured. A Stryker 800 autopsy saw was then used

to remove the bullae and cochlear windows. The ossciles were disarticulated from the bullae. Periotic bone samples were separated from the tympanic in some cases and preserved in a ten percent formalin solution for future studies.

2.4 Ossicular weight determination

Ossicles were cleaned thoroughly using scalpels and forceps to remove all surrounding tissue and membranes prior to weighing. Weights were taken as soon after disarticulation as possible to ensure that weights reflected moist, peri-normal physiological conditions as much as possible. Ossicles were weighed using an Ohaus GT410 balance (capacity 410g, readability 0.001g, linearity ±0.002g, repeatability ±0.0002g) and a Denver Instruments Company A-200DS balance (capacity 200g, readability 0.0001g, linearity ±0.0001g, repeatability ±0.0001g).

2.5 Ossicular density determination

A method based on Archiemedes's Principle refined by Lees was used to determine bone density (Lees *et al.* 1983, Lees *et al.* 1996, Lees 2001). Density determinations were carried out two days to five years following dissection (Table 4). On receipt for this study, bones were stored in a 0.2% solution of formalin to maintain hydration. The accuracy of the method was calibrated using three materials of known density: silicon carbide powder (density 3.11 g/cm^3-obtained from the WHOI Department of Geology and Geophysics); quartz (density 2.65 g/cm^3-obtained from the WHOI Department of Geology and Geophysics); and borosilicate glass (density 2.23 g/cm^3-Kimble-Kontes, type KG-

Table 4
Bone density specimens

Common name (Species name)	ID	Age class	Specimen number	Place of origin	Storage (duration)	Hydration	Hydration (duration)
Human (*Homo sapiens*)	NA	NA	NA	MEEI	Dry (1-2 years)	0.2% formalin	2 months
Northern elephant seal (*Mirounga angustirostris*)	MA23	A	F	MMC	0.2% formalin	0.2% formalin	2 days
	MA07	J	NA	MMC	L: wet	Water	1 month
						10 % formalin	1 week
						0.2% formalin	1 month
					R: dry	Water	1 month
					(14 months)	10 % formalin	1 week
						0.2% formalin	1 month
	MA13	J	NA	MMC	L wet	0.2% formalin	1 month
					R wet	0.2% formalin	1 week
					R TB	0.2% formalin	1 month
	MA14	W	NA	MMC	Wet	Water	1 month
						10 % formalin	1 week
						0.2% formalin	1 month
Pacific walrus (*Odobenus rosmarus*)	Or2H001	J	M	Ronald Kastelein	Dry (5 years)	Water	1 month
						10 % formalin	1 week
						0.2% formalin	1 month
California sea lion (*Zalophus californianus*)	ZaC19	A	F	MMC	Wet	Water	1 month
						10 % formalin	1 week
						0.2% formalin	1 month
	ZaC21	A	F	MMC	Wet	Water	1 month
						10 % formalin	1 week
						0.2% formalin	1 month
	ZaC 22	A	F	MMC	Wet	Water	1 month
						10 % formalin	1 week
						0.2% formalin	1 month
	ZaC 23	A	M	MMC	Wet	Water	1 month
						10 % formalin	1 week
						0.2% formalin	1 month

California sea lion (*Zalophus californianus*)	ZaC 12	J	M	MMC	Dry (1 month)	Water	1 month
						10 % formalin	1 week
						0.2% formalin	1 month
	ZaC 15	Y	M	MMC	Dry (1 month)	Water	1 month
						10 % formalin	1 week
						0.2% formalin	1 month
	ZaC 01	Y	F	MMC	R: dry (14 months)	Water	1 month
						10% formalin	1 week
						0.2% formalin	1 month
	ZaC 02	Y	M	MMC	R: dry (14 months)	Water	1 month
						10 % formalin	1 week
						0.2% formalin	1 month
	ZaC 17	SA	M	MMC	Wet	Water	1 month
						10 % formalin	1 week
						0.2% formalin	1 month
	ZaC 18	SA	M	MMC	Wet	Water	1 month
						10 % formalin	1 week
						0.2% formalin	1 month
	ZaC 20	SA	M	MMC	Wet	Water	1 month
						10 % formalin	1 week
						0.2% formalin	1 month
Harbor seal (*Phoca vitulina*)	PV 06	NA	NA	SeaWorld	Wet	0.2% formalin	1 month

Notes: A-adult ; J-juvenile; L-left; MEEI-Massachusetts Eye and Ear Infirmary; MMC-Marine Mammal Center, Sausalito, CA; NA-not available; R-right; SA-subadult; TB-tympanic bulla; W-weaner; Y-yearling;

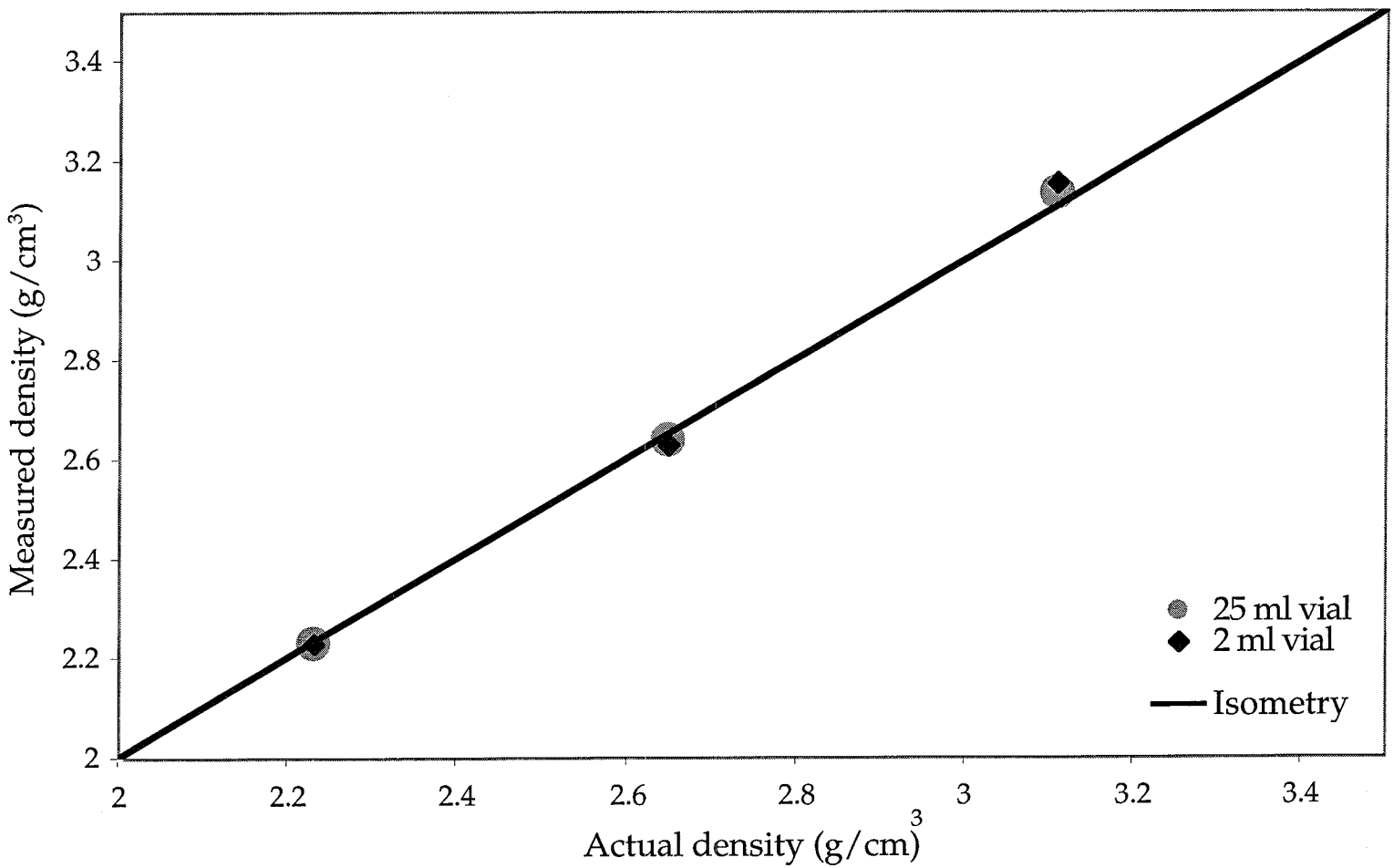


Figure 3. Calibration graph for density data. Points reflect actual values measure d for materials of known density. Measurements were taken using the same apparatus used for bone density measurements. A line reflecting isometry is plotted in relation to the data. The slope of the regression through the data was 0.995 (the plotted line is a regression for isometry, its slope falls within the 95% confidence intervals for the data regression). Standard error bars were omitted because they were smaller than the points themselves.

33 glass). In each case, the density determination was repeated four times to obtain a standard error. Figure 3 is a calibration plot for the density determination. Known density values are plotted against measured values. The regression line for the data falls within the 95% confidence intervals for isometry. Standard error bars are not shown because they would have been smaller than the plot points themselves.

2.5.1 Detailed density determination procedure

Density was determined by a volume displacement method. Specific gravity vials (25 ml Kimax Specific Gravity Vial for oils, Fischer catalog #01-710; or a 2 ml Moore-Van Slyke Specific Gravity Bottle, Fischer catalog #03-249) were used. A vial was filled with deionized water (density 1.00 g/cm^3) and weighed (Ohaus GA110 scale, capacity 110g, readability ±0.0001g, linearity ± 0.0002g, repeatability ±0.0002). The vial was then filled with deionized water and ossicles and weighed. The ossicles were removed from the vial and allowed to dry briefly. They were placed on a scale until their weight remained constant over a 60 second period to ensure a true weight. All measurements were repeated at least three times; wet measurements were repeated until three replicates within a given tolerance were obtained (1 mg for the 25 ml vial, 0.5 mg for the 2 ml vial).

2.5.2 Density determination calculations

The weight of the vial filled with water (W_w) was added to the weight of the ossicles (W_a). The weight of the vial containing water and ossicles (W_b) was subtracted from that quantity, yielding the weight of the water the ossicles displaced (W_d) (equation 1).

$(W_w + W_a) - W_b = W_d$ (1)

That weight was divided by the density of the water (1.00 g/cm^3), yielding the volume of water displaced, which is equivalent to ossicular volume. Ossicular density was obtained by dividing the ossicular weight by the ossicular volume derived from the fluid displacement measures. These calculations result in equation (2):

$$\partial_b = \frac{W_a}{(W_a + W_w - W_b)} \partial_w \quad (2)$$

Where

∂_b – bone density

W_a – bone weight in air

W_w – weight of water alone

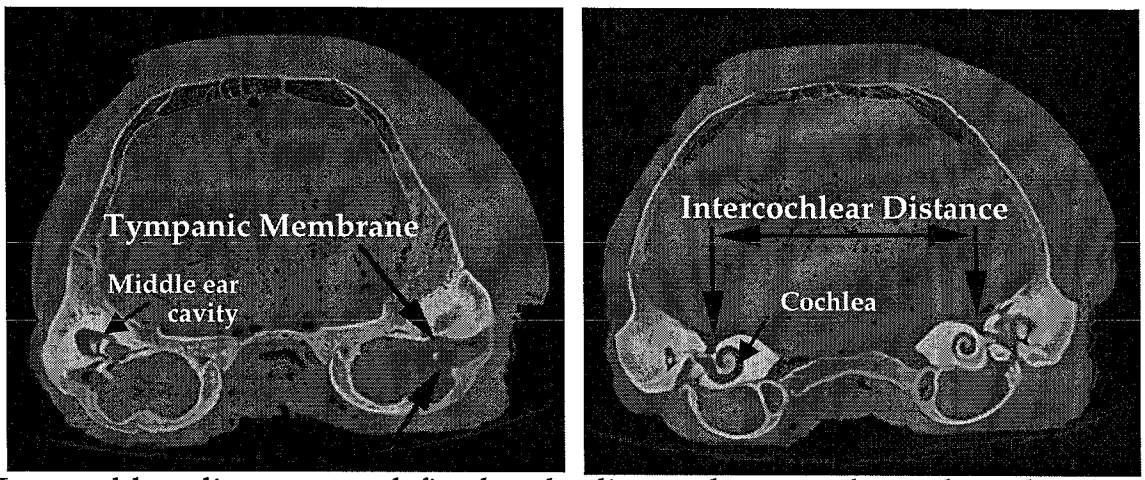
W_b – weight of water and bone

∂_w – density of water

2.6 Digital dissection: Computerized Tomography (CT scans)

All specimens in this study were scanned prior to dissection. Scan images from live animals that were used in this study were obtained previously from animals from the New England Aquarium that were scanned as a diagnostic procedure. All scans were acquired using contiguous spiral protocols on a Siemens Somatom at the Massachusetts Eye and Ear Infirmary. Scan images were re-formatted at slice intervals of 100 microns to 3 millimeters. Scan images for this study were formatted using bone settings with a window of 3500 and center of 500. Interaural distances were determined from scans using 200 mm

Mitutoyo Series 505 dial calipers (accuracy ± 0.03 mm). These measurements included intermeatal, intertympanic, and intercochlear distances (Fig. 4). Intermeatal distance was defined as the straight line distance between the meatus (the opening of the meastus at the base of the pinnae in sea lions). Intertympanic distance was measured between the superior and inferior ends of the tympanic annulus and at the umbo when all three distances were visible on CT images. The reported distance was an average of these three measurements.



Intercochlear distance was defined as the distance between the oval windows.

Figure 4. CT scans of a northern elephant seal. The tympanic membrane (left) and intercochlear distance (right) are highlighted.

Scans were also used to reconstruct three-dimensional images of the ear region in three animals. For one case (MA11), these reconstructions were generated using the software on-line on the Siemens Somatom scanner. For two others (PV06, ZaC18), images were transferred to an Ultra 10 Elite 3D SUN Microsystems workstation. Three-dimensional reconstructions were obtained using an interactive segmentation tool, MRX (SPL version 4.0, August 1998; GE

Medical Systems, Milwaukee, WI and the Surgical Planning Laboratory, Brigham and Women's Hospital, Boston, MA), and a three-dimensional reformatting program, 3D Slicer (the Surgical Planning Laboratory Brigham and Women's Hospital, Boston, MA).

3. Results

3.1 Outer ear

Interaural distance measurements are presented in Table 5. Separate values are provided for adult males and females to evaluate whether there is any auditory system sexual dimorphism and, if so, to consider this in measurement interpretations. This possibility was based on previous work which has established that grossly California sea lions and northern elephant seals exhibit a strong degree of sexual dimorphism (Daugherty 1965; Bartholomew 1967; Briggs & Morejohn 1975). Harbor seals, in contrast, show very little sexual dimorphism (Allen 1902; Daugherty 1965; Shaughnessy & Fay 1977). Distances from the tip of the snout to the meatus/base of pinnae are also provided (Table 5).

Interaural distance measurements were converted into maximal Interaural Time Distances (IATD) for both air and water (Table 6). This calculation involved dividing the measured interear distance by the speed of sound in the appropriate media. In this case 350 m/s was used for the speed of sound in air, while 1450 m/s was used for water. This yields the time required for sound traveling as a plane wave arriving at one ear to reach the opposite ear. Scientists have established a correlation between IATD and functional upper frequency hearing limit in terrestrial mammals (Masterton *et al.* 1969, Heffner & Heffner 1992). The upper functional hearing limit for terrestrial animals and pinnipeds in air was defined as the frequency perceived at threshold of 60 dB re 20 µPa (Fay 1992, Hemilä *et al.* 1995) (Fig 5). One hundred and twenty dB re 1 µPa was used for cetaceans in water; this convention corresponds to the underwater intensity equivalent of the in-air limit (Ketten 2000).

Table 5
Interaural distance measurements

Common name *Species name*	Sex	Age class	Mean external inter-meatal Distance cm (range)	Mean internal inter-meatal/distance Cm (range)	Mean inter-tympanic distance (cm) (range)	Mean inter-cochlear distance (cm) (range)	Mean distance snout-left meatus(cm) (range)	Mean distance snout-right meatus (cm) (range)
California Sea lion *Zalophus californianus*	3F	A	19.8 (18.7-21.6)	14.6 (13.9-16.0)	6.54 (6.11-9.96)	5.93 (5.58-6.50)	18.3 (16.6-20.0)	18.5 (10.1-19.1)
	10M	A	31.1 (30.0-32.4) (n=2)	29.3 (20.6-32.8)	6.93 (n=1)	6.44 (n=1)	24.6 (n=1)	25.0 (n=1)
	3M	Sa	20.5 (20.8-20.2)	15.0 (14.7-15.3)	6.81 (6.67-6.97)	6.36 (6.28-6.47)	21.0 (20.2-21.0)	20.8 (19.5-22.1)
	1M	J	20.1	14.2	7.2	6.6	19.7	19.2
	1M	Y	17.9	13.0	6.04	5.72	16.8	16.9
Northern elephant seal *Mirounga angustirostris*	1F	A	27.2	21.4	13.0	10.7	26.7	27.5
	1M	A	NA	NA	20.66	13.48	NA	NA
	4F, 3NA	J,Y, W	17.2 (10.1-21.5)	15.6 (13.1-17.4)	8.76 (5.03-10.09)	7.00 (3.62-8.83)	17.78 (11.10-21.30)	17.48 (10.30-12.60)
Harbor seal *Phoca vitulina*	1F,3M	A	16.1 (15.1-17.3)	13.9 (13.0-15.1)	8.42 (n=1)	7.92 (n=1)	16.5 (n=1)	17.0 (n=1)
	3F,3M 3NA	J	14.3 (11.8-17.5)	11.7 (10.7-14.1)	7.82 (7.18-8.36)	7.24 (6.26-7.92)	12.3 (n=1)	13.3 (n=1)
	1F,5M 4NA	Y	12.8 (9.7-10.8)	10.4 (9.7-10.8)	7.53 (6.78-8.45)	6.69 (5.52-7.92)	NA	NA
	2NA	P	NA	NA	6.75 (6.72-6.78)	5.72 (5.52-5.92)	NA	NA

Notes: A-adult; F-female; J-juvenile; M-male; n=x -data available from x specimens; NA-not available; P-pup; Sa-sudadult; W-weaner; Y-yearling;

Table 6
IATD Data

Common name (Species name)	Specimen number	Age class	Mean External Intermeatal IATD (µsec) air (water)	Internal Intermeatal IATD (µsec) air (water)	Inter-tympanic IATD (µsec) air (water)	Inter-cochlear IATD (µsec) air (water)	Hearing limit in air (kHz)	Hearing limit in water (kHz)
California sea lion (*Zalophus californianus*)	F	Adult	565.7 (136.6)	417.1 (100.7)	186.9 (45.1)	169.4 (40.9)	32 †	36 ‡
	M	Adult	888.6 (214.5)	837.1 (202.7)	198.0 (47.8)	148.0 (44.4)		
		Subadult	585.7 (141.4)	428.6 (103.5)	194.6 (46.97)	181.7 (43.86)		
		Juvenile	574.3 (138.6)	405.7 (97.93)	205.7 (49.66)	188.6 (45.52)		
		Yearling	511.4 (123.5)	371.4 (89.66)	172.6 (41.66)	163.4 (39.45)		
Northern elephant seal (*Mirounga angustirostris*)	1F	Adult	777.1 (187.6)	611.43 (147.6)	371.43 (89.66)	305.7 (73.79)	28 Ω	55 Ω
	1M	Adult	NA	NA	590.3 (92.97)	385.1 (142.5)		
	4F	Juvenile	491.4 (118.6)	445.7 (107.6)	250.3 (60.41)	200 (48.28)		
	3NA	Yearling Weaner						
Harbor seal (*Phoca vitulina*)	1F	Adult	443.60 (107.7)	387.1 (93.5)	240.5 (58.1)	226.4 (54.62)	20 ∂	64 ∂
	3M	Adult	465.2 (112.3)	400.5 (96.67)	NA	NA		
	3F,3M, 3NA	Juvenile	408.6 (98.6)	334.3 (80.7)	223.4 (53.93)	206.9 (49.93)		
	1F, 5M, 4NA	Yearling	365.7 (88.28)	71.7 (71.7)	215.1 (51.93)	191.1 (46.14)		
	2NA	Pup	NA	NA	192.9 (46.55)	113.4 (39.45)		

Notes: F-female; M-male; NA-not available; † -Schusterman 1974; ‡ -Schusterman *et al* 1972; Ω -Kastak & Schusterman 1999; ∂ -Møhl 1968a;

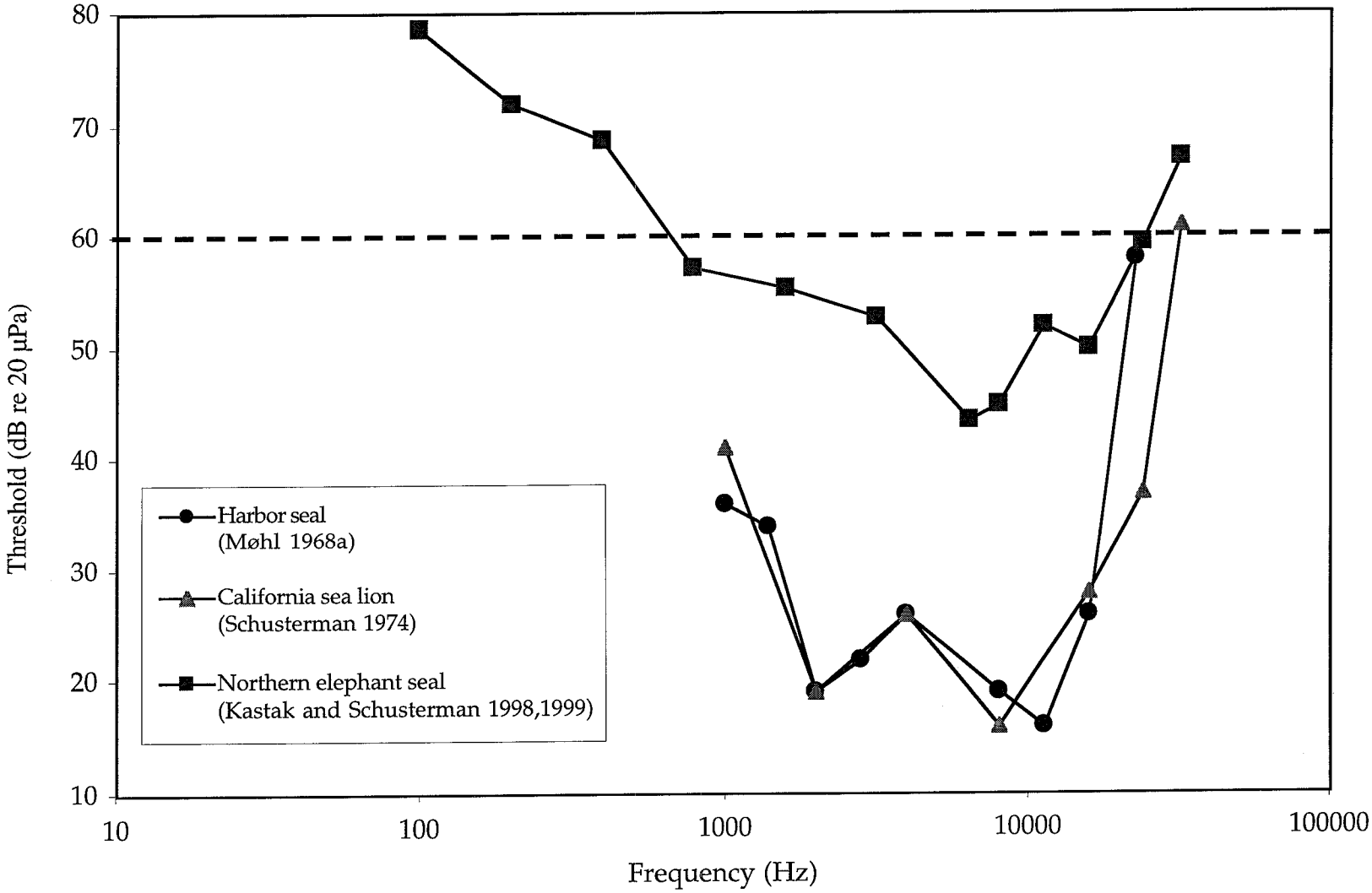


Figure 5. Pinniped aerial audiograms. The dashed line at 60 dB represents the functional in-air hearing limit.

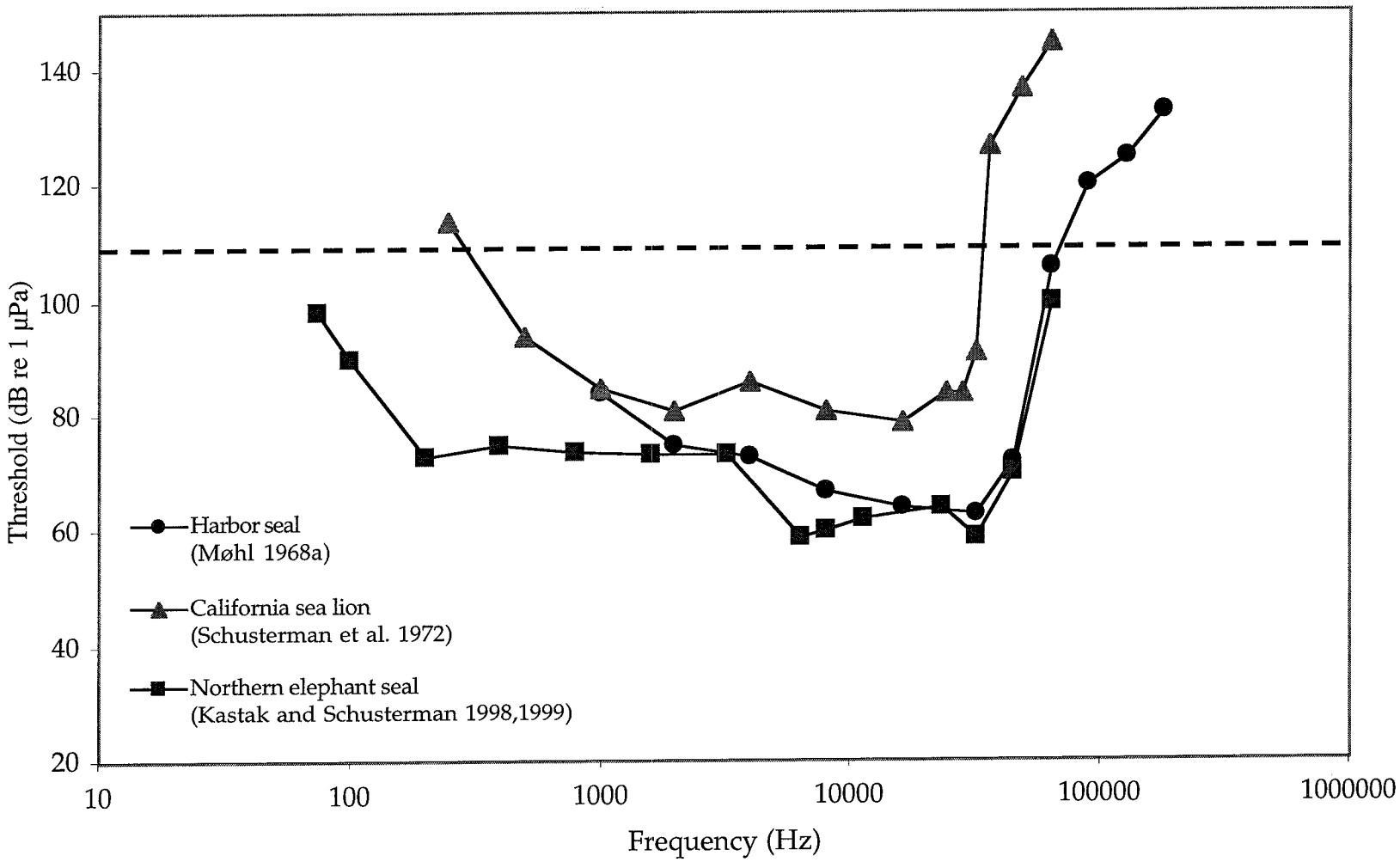


Figure 6. Pinniped underwater audiograms. The dashed line at 100dB represents the functional underwater threshold for pinnipeds.

One hundred and ten dB re 1 µPa was used for the functional hearing limit for pinnipeds in water (Fig 6). This limit was selected to coincide with the harbor seal upper frequency limit (Møhl 1968a). It lies in the middle region of the steepest slope of the harbor seal audiogram, prior to a second shallower slope (from 90-180 kHz). The human functional upper frequency limit lies in a similar location midway along a steep slope in our audiogram. Møhl (1968a) hypothesized that the second, shallower slope, not observed in land mammals at audible frequencies, represented a transition from the more sensitive sound reception pathway through the outer and middle ear to bone conduction (Fig 6). Figure 7 illustrates IATD vs. functional upper frequency hearing limit for the pinniped species in this study. Four types of interaural measurements, curvilinear intermeatal time distances in both air (air, filled circles) and water (water, open circles), straight-line intermeatal time distance in water (im-water, squares), and an average of the intercochlear and intertympanic time distances (ic/t water, triangles) were plotted with regression lines from prior studies (Heffner & Heffner 1992, Ketten 2000). Note that the curvilinear in water and straight line intermeatal and intercochlear/intertympanic distances were calculated using the sound speed values for water (1450 m/s) rather than air (350 m/s).

3.2 Middle ear

3.2.1 Ossicular chain morphology

Figures 8 and 9 show a comparison of ossicular morphology. Figure 10 shows three dimensional reconstructions of the ossicles *in situ* from scans. As these

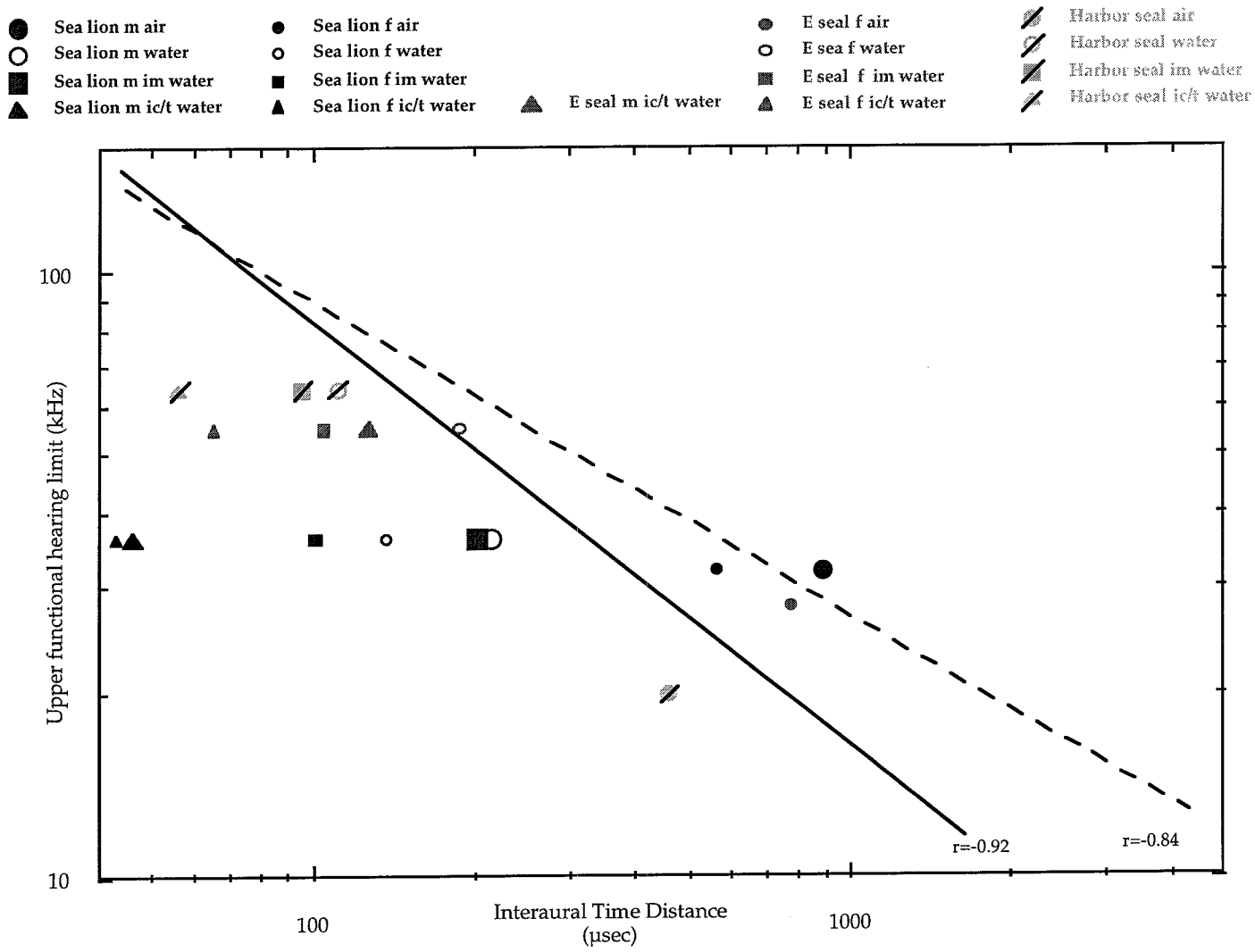


Figure 7. IATD vs. Upper functional hearing limit. See next page for complete caption.

Figure 7. Interaural time distance vs. functional upper frequency hearing limit. In air upper functional hearing limits are defined following Fay (1992) as 60 dB re 20 µPa.; in water limits for cetaceans are defined following Ketten (2000) as 120 dB re 1 µPa; in water limits for pinnipeds are defined as 110 dB re 1 µPa. The solid line is a regression from previously published data for aquatic mammals, actual data points omitted (Ketten 2000). Terrestrial mammal interaural distances based on intermeatal distances. Aquatic mammal interaural distances based on intercochlear distances. The dashed line is a previously published regression for terrestrial animals for in-air hearing limits, data points omitted (Heffner & Heffner 1992). Four points are plotted for each pinniped species in this study: external intermeatal time distance in air (air, filled circles) and in water (water, open circles), internal intermeatal (im water, squares) and intercochlear-intertympanic averages (ic/t water, triangles). For the highly sexually dimorphic species, California sea lions and Northern elephant seals, separate points are plotted for males and females; Sea lion- California sea lion (*Zalophus californianus*); E seal-Northern elephant seal (*Mirounga angustirostris*); Harbor seal-Harbor seal (*Phoca vitulina*);

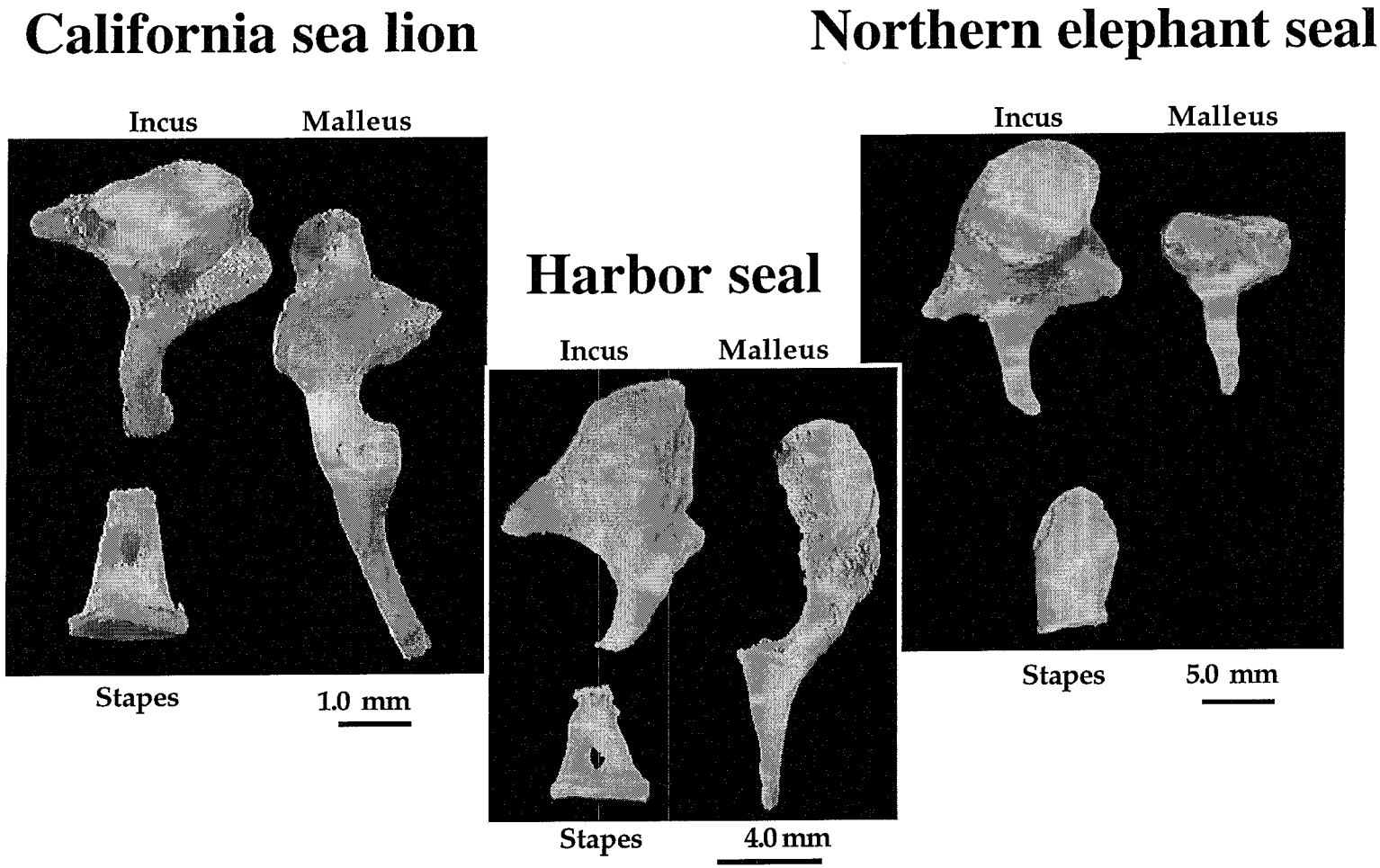


Figure 8. Ossicles from three species of pinnipeds: upper right northern elephant seal (*Mirounga angustirostris*), Middle lower harbor seal (*Phoca vitulina*), upper left California sea lion (*Zalophus californianus).* Note the difference in scales.

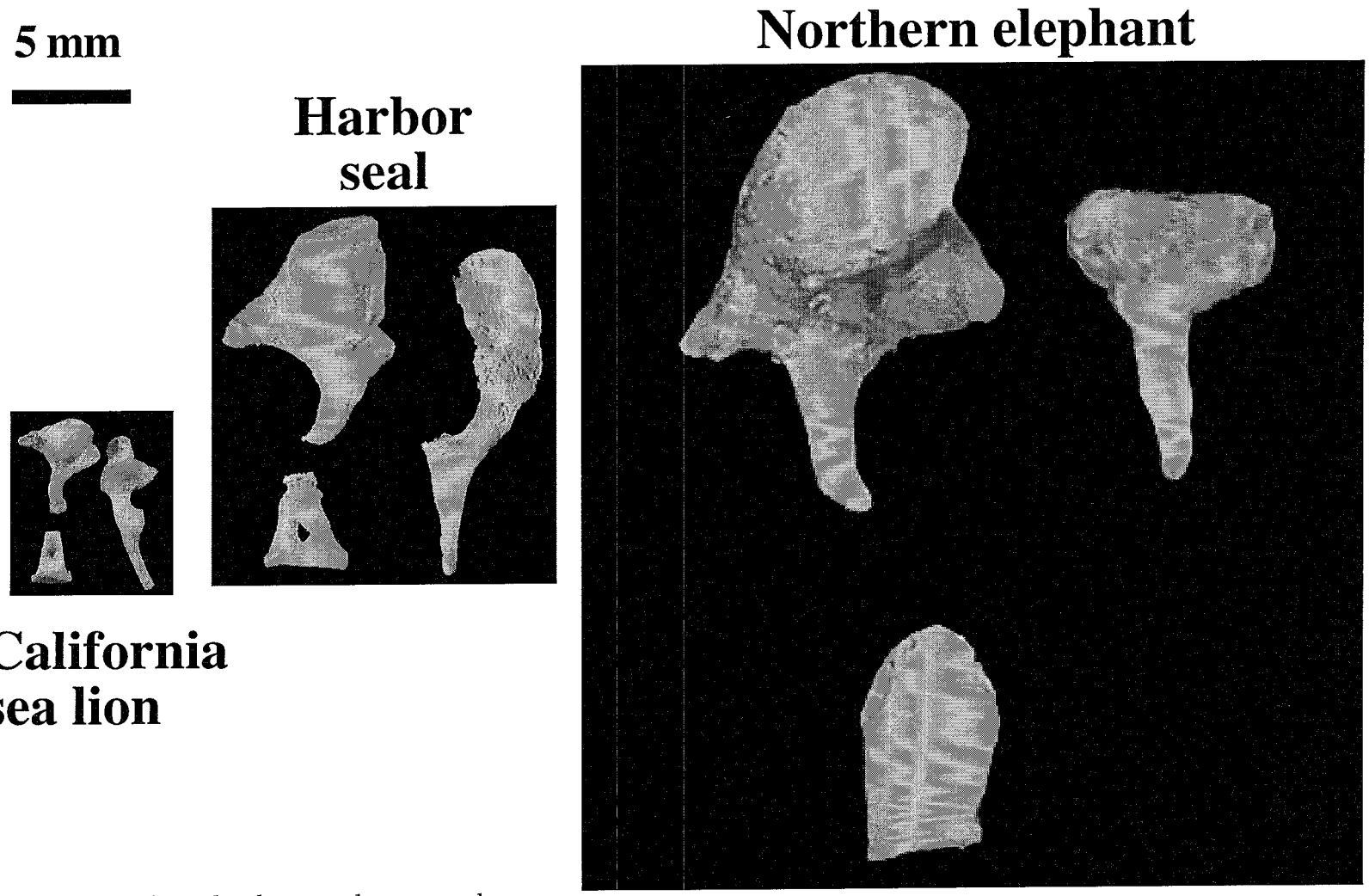


Figure 9. Pinniped ossicles shown at the same scale.

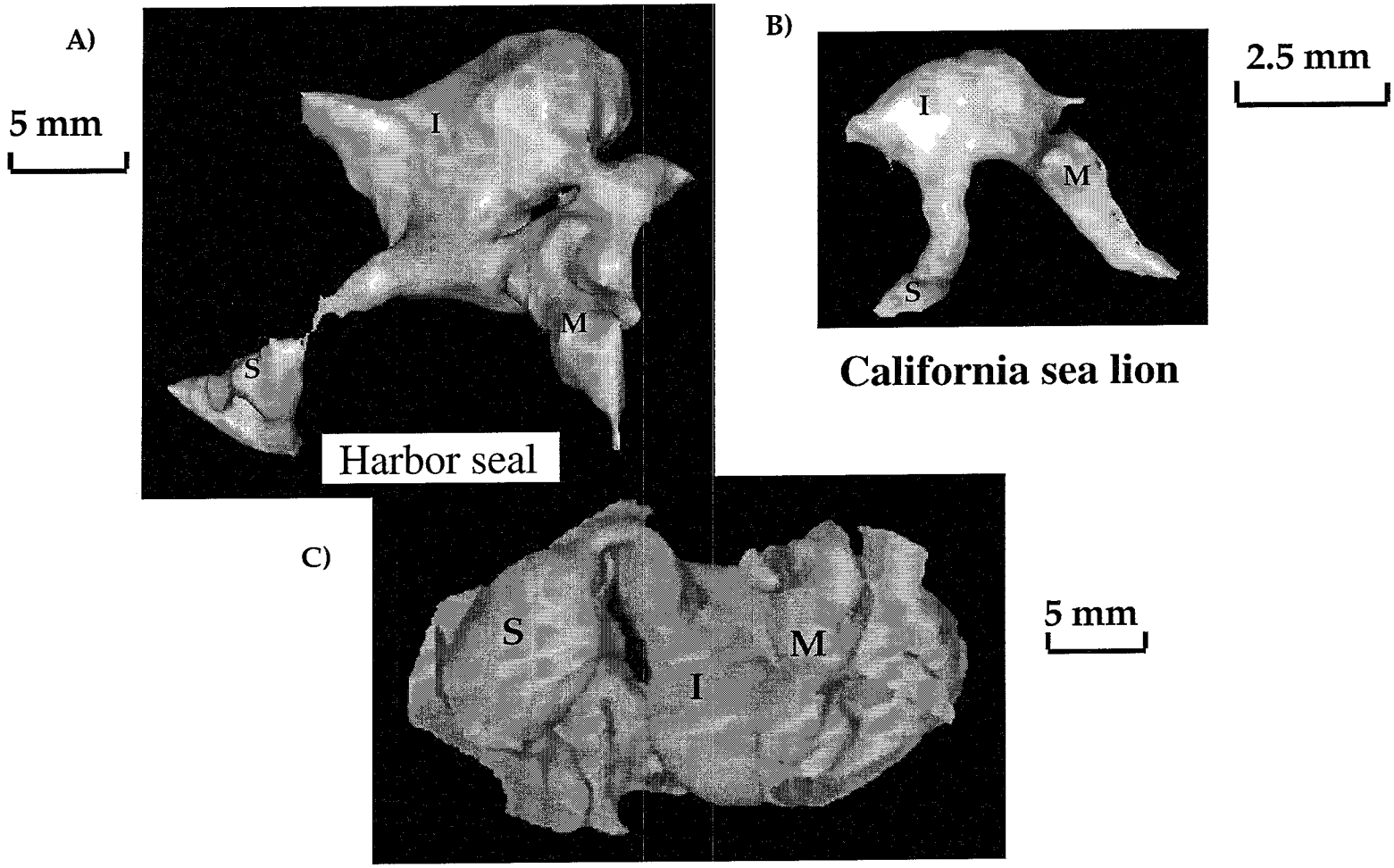


Figure 10. Three-dimensional reconstructions of CT images of the ossicular chain in situ for A) the harbor seal (*Phoca vitulina*), B) the California sea lion (*Zalophus californianus*), and C) the northern elephant seal (*Mirounga angustirostris*).

reconstructions illustrate, the ossicular chains in these pinnipeds are distinctly angled. Future work is necessary to measure the angles between the ossicles.

3.2.2 Ossicular weights

Table 7 lists mean ossicular weights obtained in this study. Past studies have indicated large weight variations for pinniped auditory ossicles (*e.g.* Ramprashad et al 1972). Table 8 compares pinniped ossicular weights with those of other mammals. Figure 11 plots the ossicular weight function (x), originally defined by Hemilä *et al* .1995 and modified for this study (Equation 3), vs. functional upper frequency hearing limit.

$$x = 1/z^{0.333} \quad (3)$$

where x = ossicular weight function and z = total weight of ossicles (mg);
The original study by Hemilä *et al.* used only the weights of the malleus and incus because stapes weights were unavailable for some specimens. The formulation used in this thesis includes the mass of the stapes as well and therefore does not presume stapes weights are a constant function of the other ossicular weights. This assumption is a good approximation for most mammals given their small stapeidal weights. However, it is not for true seals. Taking stapedial weight into account is particularly important for pinnipeds, especially in light of the unusual stapedial anatomy in the elephant seal (Fig. 8). Three regressions of differing slopes are plotted, one for terrestrial mammals in air (Eurasian hedgehog-*Erinaceus europaeus*, desert hedgehog-*Hemiechinus auritus*, common shrew-*Sorex auaneus*, common mole-*Talpa europaea*, fish-catching bat-*Noctilio leporinus*, mouse-eared bat-*Myotis myotis*, wiskered bat-*Myotis mystacinus*,

Table 7

Ossicular weight results

Common name (Species name)	ID	Age class	Number of specimens	Mean incus weight (mg) (range)	Mean malleus weight (mg) (range)	Mean stapes weight (mg) (range)	Mean combined weight (mg) (range)
California sea lion (*Zalophus californianus*)	ZaC 19, 21, 22, 23	A	3F, 1M	10.6 (9.8-12.5)	8.4 (7.5-9.4)	2.4 (2.0-3.3)	21.8 (20.0-25.1)
	ZaC 17, 18, 20	Sa	3M	12.6 (12.0-13.3)	9.0 (8.4-10.0)	2.4 (2.1-2.6)	25.1 (23.3-27.3)
	ZaC 12	J	1M	12.1 (12.0-12.1)	8.2 (8.1-8.2)	2.7 (2.1-2.6)	25.0 (23.3-26.7)
	ZaC 1,2,15	Y	1F, 2M	14.7	9.0 21.0 f	2.0 (2.0)	23.9 (23.0-25.7)
	ZaC 1, 2, 12, 15, 17, 18, 19, 20, 21, 22, 23	All	4F, 7M	11.1 (9.9-12.4)	8.3 (7.5-9.4)	2.4 (2.0-3.3)	23.2 (20.0-27.3)
Pacific walrus (*Odobenus rosmarus*)	Or2H001	J	M	99.1	97.0	29.4	225.4 n=1
Harbor seal (*Phoca vitulina*)	PV06	NA	NA	168.6	67.4	14.4	250.8 (250.3-251.3)
Northern elephant seal (*Mirounga angustirostris*)	MA 14	W	NA	668.5 (691.7.-705.3)	171.5 (164.0-179.0)	188.2 (186.3-190.0)	1058 (1055-1061)
	MA 07 MA 13	J	NA	1184.3 (1089.9-1481.7)	232.1 (207.2-280.8)	295.8 (218.9-428.5)	1902 (1668.0-2156)
	MA 23	A	F		1575 Fu (1571-1579)	291 (286-296)	1866 (1857-1875)

Notes: A-adult; F-female; Fu-fused; J-juvenile; M-male; NA-not available; n=x sample size is x; Sa-subadult; W-weaner; Y-yearling;

Table 8
Ossicular weight comparison

Common name (*Species name*)	Age class	Mean weight incus (mg)	Mean weight malleus (mg)	Mean weight stapes (mg)	Mean total weight (mg)
Human	NA	27 †	23 †	3 †	62 †
(*Homo sapiens*)	NA	33.6 ‡	28.5 ‡	2.5 ‡	62.0 ‡
California sea lion	A	10.6	8.4	2.4	21.8
(*Zalophus californianus*)	Sa	12.6	9	2.4	25.1
	J	12.1	8.2	2.7	25.0
	Y	14.7	9.0	2.0	23.9
	NA				18.0 Ω
	NA				18.0 ∂
Northern fur seal	NA		13.39 Fu ‡		13.39 ‡
(*Callorhinus ursinus*)					
Pacific walrus	J	97.0	99.1	29.4	225.4
(*Odobenus rosmarus*)					
Harbor seal	NA	168.6	67.4	14.4	250.4
(*Phoca vitulina*)	NA	123.2 ‡	40.82 ‡	10.69 ‡	174.71 ‡
	NA				204 ∂
Northern elephant seal	A		1575	291	1,866
(*Mirounga angustirostris*)	J	1184.3	232.4	295.8	1,712.5
	W	702	164	186	1,058
Harp seal	NA	135 Ω	62 Ω	30.5 Ω	227 Ω
(*Phoca groenlandica*)	NA	132.8 ‡	68.03 ‡	14.89 ‡	215.72 ‡
	NA	135 §	62 §	30.5 §	227.5 §
Bearded seal	NA	116.0 ‡	67.26 ‡	21.54 ‡	204.8 ‡
(*Erignathus barbatus*)					
Grey seal	NA	214.9 ‡	87.51 ‡	14.89 ‡	318.24 ‡
(*Halichoerus grypus*)					
Ringed seal	NA	111.7 ‡	35.52 ‡	11.15 ‡	158.37 ‡
(*Phoca hispida*)					
Bowhead whale	NA	529 ¶	1,183 ¶	169 ¶	1,881 ¶
(*Balaena mystcetus*)					
Northern Atlantic right whale	A	1,687 ß	665 ß		2352 ß
(Eubalaena glacalis)					
Fin whale	A	510 ¥	1,243 ¥	278 ¥	2,031 ¥
(*Balaenopter physalus*)	Fe	1,360 ¥	3,180 ¥	590 ¥	5,130 ¥
Bottlenose dolphin	NA	32.6 ¶	135.4 ¶	8.9 ¶	176.9 ¶
(*Tursiops truncatus*)					
Risso's dolphin(*Grampus griseus*)	NA	50.0 ¶	207.5 ¶	18.4 ¶	275.9 ¶
Dall's porpoise(*Phocoenoides dalli*)	NA	18.4 ¶	71.7¶	9.8 ¶	99.9 ¶
Harbor porpoise (*Phocoena phocoena*)	NA	15.9 ¶	64.9 ¶	8.6 ¶	89.4 ¶
Common dolphin (*Delphinus delphis*)	NA	19.7 ¶	81.1 ¶	5.2 ¶	106.0 ¶
Killer whale (*Orcinus orca*)	NA		772.9 ¶	49.8 ¶	822.7 ¶

Notes: A- adult; Fe-Fetal; Fu-Fused; J-juvenile; NA-Not Available; Sa-Subadult; W-Weaner; Y-Yearling; † - Schuknecht 1993; ‡ -Nummela *et al.* 1995; Ω -King 1983; ∂ -Møhl 1968b; § - Ramprashad *et al.* 1972; ¶ - Norris and Leatherwood 1981; ß - Parks pers. comm.; ¥ - Lees *et al.* 1996;

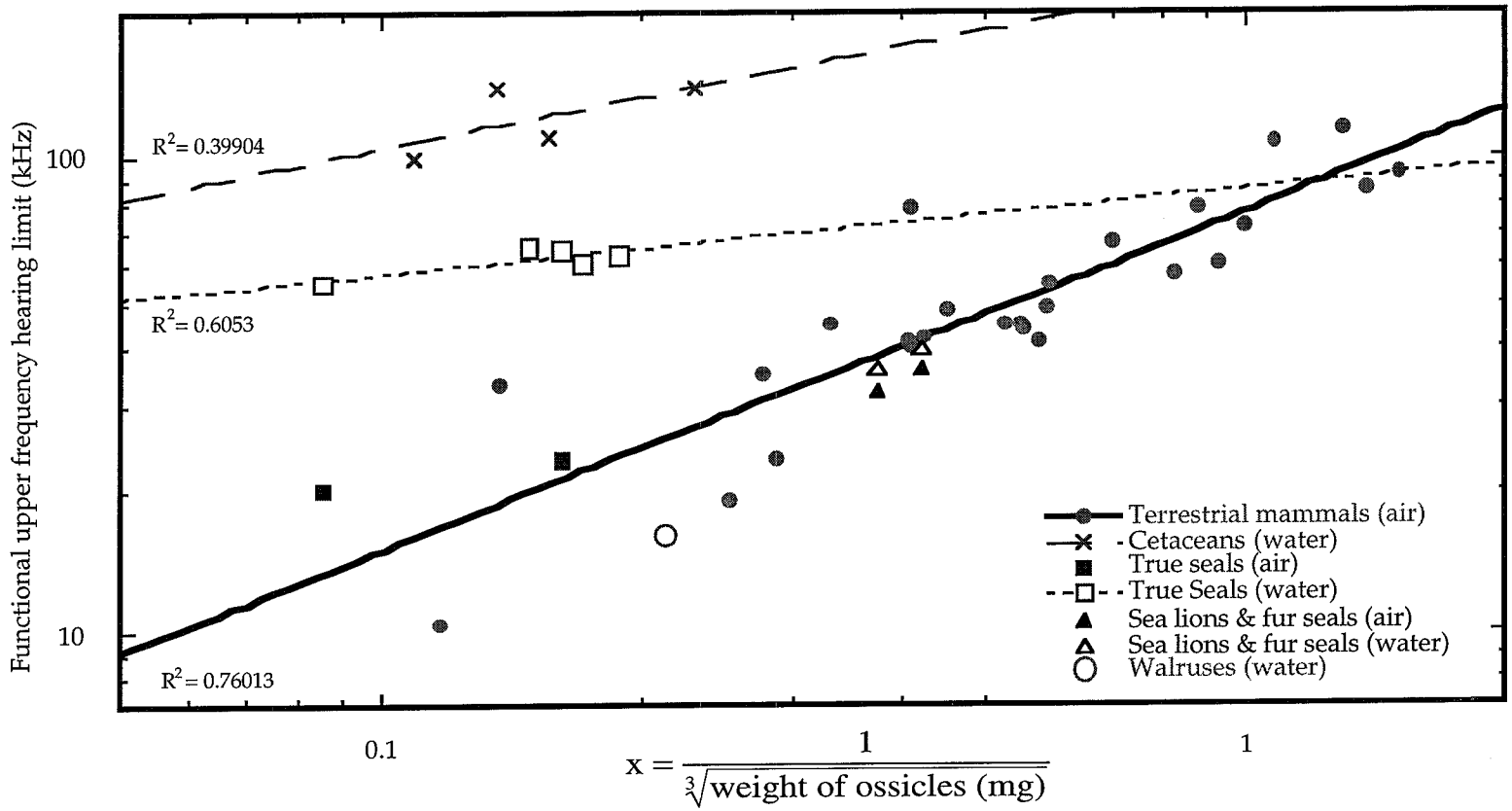


Figure 11. Functional upper frequency hearing limits vs. a function of ossicular weight (modified from Hemilä et al 1995) . Hearing thresholds defined as 60 dB re 20 µPa. (air-terrestrial mammals and pinnipeds); 90 dB re 1 µPa (water-pinnipeds) and 120 dB re 1 µPa (water-cetaceans). True seals in water include: the grey seal, (*Halichoerus grypus*), the harp seal (*Phoca groenlandica*), and the ringed seal (*Phoca hispida*); true seals in air and in water include: the harbor seal (*Phoca vitulina*), and the northern elephant seal (*Mirounga angustirostris*); sea lions and fur seals include the California sea lion (*Zalophus californianus*) and the northern fur seal (*Callorhinus ursinus*); the walrus in water datum consists of one measure from a Pacific walrus (*Odobenus rosmarus*). (Additional data compiled from Hemilä et al 1995, Kastak & Schusterman 1999, Kastelein pers. comm., Ketten 2000, Møhl 1968a, Moore & Schusterman 1987; Norris & Leatherwood 1981, Terhune & Ronald 1972, 1975).

Brandt's bat-*Myotis brandti*, slow loris-*Nycticebus coucang*, potto-*Perodicticus potto*, bushbaby-*Galago senegalensis*, squirrel monkey-*Saimiri sciureus*, owl monkey-*Aotus trivirgatus*, blue monkey-*Ceropithecidae mitis*, yellow baboon-*Papio cyncephalus*, chimpanzee-*Pan troglodytes*, human-*Homo sapiens*, rabbit-*Oryctolagus cuniculus*, mountain hare-*Lepus timidus*, squirrel-*Sciurus vulgaris*, pocket gopher-*Geomys bursarius*, beaver-*Castor fiber*, grasshopper mouse-*Onychomys leucogaste*, cotton rat-*Sigmodon hispidus*, gerbil-*Meriones unguiclatus*, common vole-*Microtus arvalis*, muskrat-*Ondatra zibethica*, black rat-*Rattus rattus*, laboratory rat-*Rattus norvegicus*, feral house mouse-*Mus musculus*-wild, laboratory mouse-*Mus musculus*-lab, guinea pig-*Cavia porcellus*, chinchilla-*Chinchilla langier*, fox-*Vulpes vulpes*, racoon dog-*Nyctereutes procyono*, wolf-*Canis lupis*, dog-*Canis familaris*, brown bear-*Ursus arctos*, least weasel-*Mustela nivalis*, European mink-*Mustela lutreola*, American mink-*Mustela vison*, glutton-*Gulo gulo*, badger-*Meles meles*, otter-*Lutra lutra*, sea otter-*Enhydra lutris*, cat-*Felis catus*, lynx-*Felis lynx*, lion-*Panthera leo*, Indian elephant-*Elephas maximus*, horse-*Equus caballus*, pig-*Sus scrofa*, camel-*Camelus bactrianus*, elk-*Alces alces*, reindeer-*Rangifer tarandus*, cattle-*Bos taurus*, musk ox-*Ovibos moschatus*, and sheep-*Ovis aries*), one for true seals in water (grey seal-*Halichoerus grypus*, harp seal-*Phoca groenlandica*, ringed seal-*Phoca hispida*, harbor seal-*Phoca vitulina*, and northern elephant seal-*Mirounga angustirostris*.) and a third for cetaceans in water (bottlenosed dolphin-*Tursiops truncatus*, killer whale-*Orcinus orca*, harbor porpoise-*Phocoena phocoena*, and Risso's dolphin-*Grampus griseus*).

The pinniped regression lies between the aquatic and terrestrial regressions, suggesting an anatomical compromise between hearing in the two environments.

3.3.3 Ossicular densities

Table 9 lists density values obtained in this study. While Table 10 shows these values in the context of other aquatic and terrestrial mammals. Figure 12 graphs ossicular density vs. function upper frequency hearing limit. Note the trend in the aquatic mammal data of increasing density with increasing upper frequency hearing limit, similar to that found by Parnell and Dreher (1963) in a mixed set of aquatic and terrestrial mammals.

Table 9
Ossicular density results

Common name *Species name*	ID	Age class	Sex	Density g/cm^3
Human	NA	NA	NA	2.16 ±0.01
Homo sapiens				2.15 I
				2.16 Ma
California sea lion	ZaC 19, 21, 22, 23	Adult	3F, 1M	2.26
Zalophus californianus	ZaC 17, 18, 20	Subadult	3M	2.22
	ZaC 1, 2, 12, 15	Juvenile, Yearling	1F, 3M	2.22
	ZaC 1, 2, 12, 15, 17, 18, 19, 20, 21, 22, 23	All	4F, 7M	2.22
	ZaC 18, 20	Subadult	2M	2.05 TB
	ZaC 18	Subadult	M	2.02 TB
	ZaC 20	Subadult	M	2.03 TB
Pacific walrus	Or2H001	Juvenile	M	2.29
Odobenus rosmarus				
Harbor seal	PV06	NA	NA	2.43
Phoca vitulina				2.24 TB
Northern elephant seal	MA 07	Juvenile	NA	2.34
Mirounga	MA 13	Juvenile	NA	2.31
Angustirostris				2.12 TB
	MA 13, 14	Juvenile	NA	2.31
	MA 14	Weaner	NA	2.07
	MA 23	Adult	F	2.33 ± 0.01
				2.32 P

Notes: F-female; I-Incus; M-male; Ma-Malleus; NA-not available; P --periotic; TB-tympanic bulla;

Table 10
Ossicular density comparison

Species	Common name	Age class	Density g/cm^3 (this work)	Density g/cm^3 (other source)
Homo sapiens	Human	NA	2.16 ±0.01	2.24 †
			2.15 I	2.23 I †
			2.16 Ma	2.24Ma † 1.98Ma ‡
Cavia porcellus	Guinea pig			2.22 I ‡
Zalophus californianus	California Sea lion	Adult	2.26	
Zalophus californianus	California Sea lion	Subadult / Juvenile/ Yearling	2.22 ± 0.01 2.03 TB	
Odobenus rosmarus	Pacific Walrus	Juvenile	2.29	
Phoca vitulina	Harbor seal	NA	2.43 2.24 TB	2.2 Ω
Mirounga angustirostris	Northern elephant seal	Weaner	2.07	
Mirounga angustirostris	Northern elephant seal	Adult/ Juvenile	2.33 ± 0.01 2.32 P 2.12 TB	
Eschrichtius robustus	Gray whale			2.23 S ‡
Eubalaena glacalis	Northern Atlantic right whale			2.30 ¥
Balaenoptera physalus	Fin whale			2.49 § 2.52 TB §
Globicephala macrorhynchus	Pilot whale			2.60 I ‡
Tursiops truncatus	Bottlenose dolphin			2.69 TB § 2.62 I ‡
Stenella attenuata	Spotted dolphin			2.64 ‡

Notes: I-Incus; Ma-Malleus; NA-not available; P-periotic; S-Stapes; TB-tympanic bulla; † Kirikae 1960; ‡ Parnell & Dreher 1963; Ω Møhl 1968b; ¥ Parks pers. comm.; § Lees 2001;

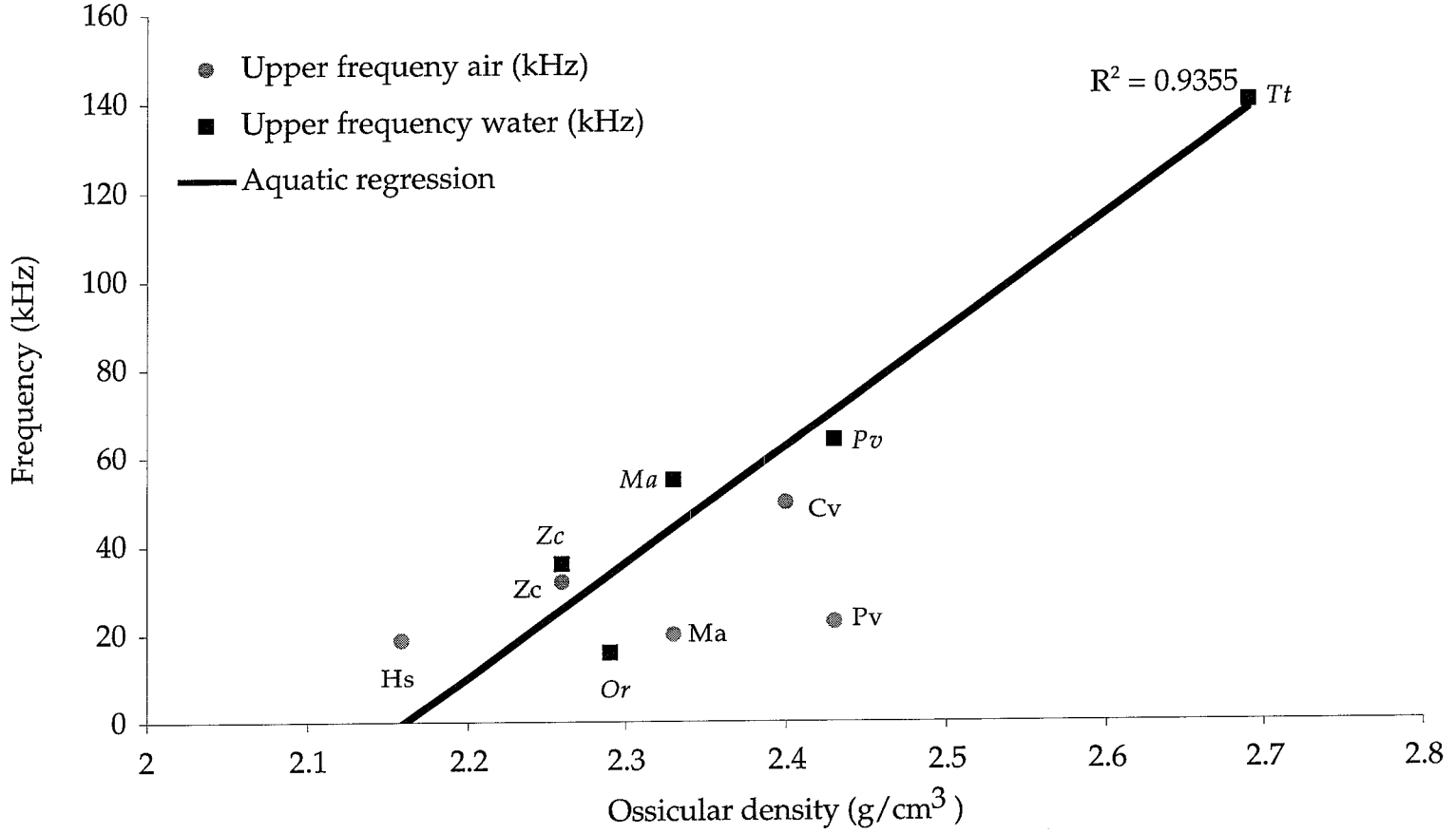


Figure 12. A comparison of ossicular density vs. functional upper frequency hearing limit. Hearing limits in air represent five species (human-*Homo sapiens*, Hs; guinea pig-*Cavia porcellus*, Cp; California sea lion-*Zalophus californianus*, Zc; harbor seal-*Phoca vitulina*, PV; and northern elephant seal-*Mirounga angustirostris*, MA;). While hearing limits in water are represented another set of five species (Pacific walrus-*Odobenus rosmarus*, *Or*; California sea lion-*Zalophus californianus*, *Zc*; harbor seal-*Phoca vitulina*, *Pv*; northern elephant seal-*Mirounga angustirostris*, *Ma*; and bottlenose dolphin-*Tursiops truncatus*, *Tt*). A regression is plotted for aquatic species in water. (Data complied from Nummela et al 1995, Kastelein pers. comm., Parks pers. comm.; Parnell & Dreher 1963.)

4. Discussion

4.1 The outer ear: Interaural Time Distance (IATD)

Masterton *et al.* (1969) proposed that IATD and upper functional hearing threshold were inversely correlated. Mammalian sound reception is limited to frequencies with wavelengths smaller than the distance between an animal's sound receptors. Masterton *et al* theorized that since small head size constrains interaural distance, it would limit low frequency sound reception and its use in localization. This put evolutionary pressure on species with small interaural distances to develop better high frequency hearing. The dashed line in figure 7 represents a regression based on terrestrial animals hearing in air (Heffner & Heffner 1992). In this case interaural distance is based on curvilinear intermeatal distance. The solid line represents a regression for fully aquatic mammals, *i.e.* cetacean hearing (Ketten 2000), with interaural distance based on intercochlear distance.

Pinnipeds hear in both media, therefore, it remains uncertain which distances are functionally important to this amphibious group. To address this question four interaural distance types are plotted for the three pinniped species examined here. These interaural time-distances included the external curvilinear distance between the auditory meatuses/base of the pinnae both in air and in water, the straight line distance between the auditory meatuses/base of the pinnae in water, and the average of the straight line distance between the tympanic membranes and the straight line distance between the cochlea in water. Both male and female sea lion external curvilinear intermeatal time distances in

air lie close to the land mammal curve. The female northern elephant seal in air value also falls close to this line, lying between the terrestrial and aquatic curves. The harbor seal in air point falls near and slightly below the aquatic curve. These results suggest that all three species of pinnipeds examined utilize their auditory canals when hearing in air. The external intermeatal values for underwater hearing for all species are reasonable fits for the fully aquatic ear regression. Internal intermeatal values fall below the aquatic ear regression for underwater hearing, and they are not very good fits. The male elephant seal intertympanic-intercochlear point falls near the aquatic regression; but there is no consistent fit for the whole intercochlear-intertympanic data set. These results suggest that all three species utilize their auditory canals or some canal-parallel tissue for underwater sound reception Møhl & Ronald (1975) found similar results in an interaural distance study using cochlear microphonics. The data set in this thesis contains only intercochlear/ intertympanic points for the male northern elephant seal, however the female data set coupled with the single male measurements suggest that this species' ears are largely adapted for aquatic hearing, making intercochlear distances potentially functionally important in larger elephant seals.

Other techniques have been applied to determine the functional interaural distance in pinnipeds. Both Møhl (1968b) and Terhune (1974) used minimal angle data to address this question in harbor seals. Both found that the functional interaural distance was the same in both air and water. Møhl and Ronald (1975) addressed this question in harp seals using cochlear microphonic

measures. They found diminished responses in cochlear potentials when the meatal region of the seal's head was covered. Kastak & Schusterman (1999) argued that the evolutionary pressure imposed by deep diving would lead to the discontinued used of air borne pathways (such as the auditory canals) in northern elephant seals. While their canals are very narrow (1-2 mm), IATD data suggest that they are continuing to use their canals or paths paralleling the canals for localization. The upper frequency hearing limit is similar to those found in other true seals, however their hearing curve is shifted upwards 20 to 30 dB re 20 μPa compared to other seals. This suggests that while northern elephant seals have diminished hearing capabilities in air, there is pressure to maintain at least minimal hearing and localization capabilities on land.

4.2 Middle ear

4.2.1 Ossicular chain morphometry

In situ CT three-dimensional reconstructions of pinniped ossicular chains (Fig. 6) reveal complex structures with many twists and turns. It is likely that this intricate structure is functional and important both in air and underwater sound conduction. That is, it is unlikely that bone conduction or direct air oscillation are the primary conduction paths although bone conduction remains probable at high sound pressure and intensity levels.

4.2.2 Ossicular weight

Pinniped ossicular weights vary a great deal within species. For example, adult California sea lions varied up to 26 percent (37 percent over all age classes), while juvenile northern elephant seals varied up to 29 percent (104 percent over

all age classes). Similar variation of up to 56 percent has been shown by Ramprashad *et al.* (1972,1973) in harp seals. Reported values from different sources for other pinniped species also show a great deal of variation. For example, reported values for harbor seals vary by 43 percent. Some of this variation may be due to the condition of the specimens, with fresh bones weighing more than dried ones (Less 2001). For example, in a pilot project for this study, two sets of California sea lion ossicles that were extracted and stored in air lost 21% of their original weight over an 18 month period. However, there was no apparent systematic variation in the losses observed. Large variations are to be expected in juveniles versus adults and possibly in females versus males for some species; however pathology and nutrition are also important factors to consider. For example, Mills (1993) found 41 percent variation in ossicular weight in a study of humans receiving cortical bone grafts.

Figure 6 plots functional upper frequency hearing limit versus a function of ossicular weight (based on Hemilä *et al.* 1995). All in-air data for pinnipeds fall near the terrestrial mammal regression line. In-water data for the otariids (sea lions and fur seals) fall along the terrestrial regression, indicating mid-frequency range adapted aquatic hearing. In-water data for the phocids (true seals) is sufficiently distinct from the terrestrial regression to warrant an independent regression, and is consistent with primarily water-adapted high frequency hearing. The data set lacks sufficient resolution to comment on finer grained distinctions. A third regression for limited cetacean data is plotted above the pinniped line. The position of this line suggests that cetaceans are more high

frequency adapted than pinnipeds. The position of the pinniped regression suggests that as a group they have achieved a compromise between aquatic and terrestrial adaptation for hearing.

Kastak & Schusterman (1999) reached similar conclusions based on behavioral data, providing independent verification for this conclusion. In-water data for walruses lie below the terrestrial line reflecting a lower frequency hearing threshold than the other pinnipeds studied which has recently been confirmed by Kastelein (pers. comm.; see also Kastelein *et al.* 1996 for in-air data).

4.2.3 Ossicular density

Adult ossicular densities were often greater than those of the youngest animals sampled. However, there was little detectable difference between adult, subadult, and juvenile values across all species tested. This mirrors the human pattern of early ossification. Humans ossicles typically attain their full density by approximately 25 months of age (Yokoyama *et al.* 1999) and maintain constant density over an individual's life, barring an illness such as otosclerosis (Tohno *et al.* 1997). Pinnipeds appear to fit the fundamental pattern for the development of ossicular density.

Parnell & Dreher (1963) suggested a correlation between upper frequency hearing limit and ossicular density in a variety of both terrestrial and aquatic mammals. Figure 8 plots this relationship including some pinniped points. A regression is plotted for aquatic/amphibious mammal data. This regression confirms a positive correlation between ossicular density and upper frequency hearing limit. The terrestrial data are more scattered. This set includes three

amphibious animals, and only two terrestrial mammals. Additional data points are required to test this conclusion in water-adapted and in refining it for air-adapted species.

It is critical to use hydrated samples to obtain accurate bone density measurements (Lees *et al.* 1983, Lees 2001). Accordingly all bones in this study were stored in hydrating conditions. Older studies of bone density often used dry specimens, which would systematically lead to artificially low density values. This is the case with one of the few studies reporting marine mammal bone densities in the literature, Parnell and Dreher (1963). Other previous studies suspended samples in water during density determinations using fine line or spider web threads. Subsequent work has shown that under these conditions water creeps up the line and can artificially inflate the density value (Lees pers. comm.). This was the case with Kirikae's (1960) measurements of human bone density. The value obtained for human bones in this thesis were between the values obtained by Parnell and Dreher (1963) and Kirikae (1960), providing additional evidence for the accuracy of the method used here.

5. Conclusions

A function of ossicular weight as modified from Hemilä *et al.* (1995), was plotted against in-air functional upper frequency hearing limit (Fig. 10). Values for the pinnipeds studied here fall close to the regression for land mammals. The in-water points for the otariids (sea lions and fur seals) also fall near the land mammal regression, suggesting their hearing systems are primarily mid-frequency adapted. The in-water points for the phocids (true seals) are well above this line, suggesting that they are adapted for higher frequency in-water hearing. The odobenid (walrus) in-water point falls well below the regression, reflecting lower frequency hearing thresholds than other pinnipeds tested.

Intermeatal distances are consistent with hearing abilities in all pinnipeds examined while intercochlear distances are not. This suggests a canal-centric system, rather than of a cochlea-centric system for reception and localization of sound.

Sea lion outer and middle ear data fall close to land mammal regressions. Current data suggest they are amphibious mid-frequency adapted. However, both the IATD data and ossicular weight function for the true seals suggests they are primarily water-adapted, especially at higher frequencies. Pinniped ossicular densities fit the mammalian pattern proposed by Parnell & Dreher (1963) of a positive correlation of density with functional upper hearing limit. Anatomical data examined here provide independent confirmation for behavioral hearing studies.

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"Every now and then, go away, have a little relaxation. For when you come back to your work your judgement will be surer; since to remain constantly at work will cause you to lose power of judgement."
-Leonardo DaVinci

References

Andersen, S., 1970. Auditory sensitivity of the harbor porpoise *Phocoena phocoena*. Investigations in Cetacea 2, 255-259.

Allen, J.A., 1902. The hair seals (family Phocidae) of the north Pacific Ocean and Bering Sea. Bull. Am. Mus. Nat. His. 16, 459-499.

Bartholomew, G.A., 1967. Seal and sea lion populations of the California islands. Proc. Symp. Biol. Calif. Islands, pp 229-244.

Barnes, L.G., Domning, D.P., Ray, C.E., 1985. Status of studies on fossil marine mammals. Mar. Mamm. Sci. 1, 15-93.

Bininda-Emonds, O.R.P., Gittleman, J.L., Purvis, A., 1999. Building large trees by combining phylogenetic information: a complex phylogeny of the extant Carnivora (Mammalia). Biol. Rev. 74 143-175.

Briggs, K., Morehohn, V.G., 1975. Sexual dimorphism in the mandibles and canine teeth of the northern elephant seal. J. Mamm. 56, 224-231.

Bullock, T.H., Ridgway, S.H., Suga, T., 1971. Acoutically evoked potentials in midbrain auditory structures in sea lions (Pinnipedia). Z. vergl. Physiologie 74, 372-387.

Daugherty, A.E., 1965. Marine mammals of California. California Department of Fish and Game, Sacramento.

Denker, A., 1899. Vergleichend-Anatomische Untersuchungen über das Gehörorgan der Säugethiere. Veit and Company, Leipzig.

Doran, A.H.G.,1878. Morphology of the mammalian ossicula auditorius. Trans. Linnean Soc., Ser. II, 371-498.

England, E.S., 1961. Physical properties of components of the tympanoperiotic bone system of *Stenella graffmani*, MLP 60-11 female 69. Lockheed-California Company Interdepartmental Communication PCS-61-43.

Fay, R.R., 1988. Hearing in vertebrates. Fay-Hill Associates, Winnetka, IL.

Fay, R.R., 1992. Structure and function in sound discrimination amoug vertebrates. In: Webster D., Fay R. (Eds.), The Evolutionary Biology of Hearing. Springer, New York, pp 229-263.

Flynn, J.J., Nedbal, M.A., 1998. Phylogeny of the Carnivora (Mammalia): Congruence vs incompatibility among multiple data sets. Mol. Phylogenet. Evol. 9, 414-426.

Graham, S.F., 1967. Seal ears. Science 155, 489.

Hemilä, S., Numela, S., Reuter, T., 1995. What middle ear parameters tell us about impedance matching and high frequency hearing. Hear. Res. 85, 31-44.

Heffner, R., Heffner, H., 1990. Hearing in domestic pigs (*Sus scrofa*) and goats (*Capra hircus*). Hear. Res. 48,231-240.

Heffner, R., Heffner, H., 1992. Evolution of sound localization in mammals. In: Webster D., Fay R. (Eds.), The Evolutionary Biology of Hearing. Springer, New York, pp 691-715.

Hyrtl, J., 1845. Vergleichend-anatomische Untersuchungen über das innere Gehörorgan des Menschen und der Säugethiere. Prag.

Johnson, C.S., 1966. Auditory thresholds of the bottlenose porpoise (*Tursiops truncatus*, Montagu). US Naval Ordance Test Station Report, NOTS TP 4178, pp 1-28.

Kastak, D., Schusterman, R.J., 1998. Low frequency amphibious hearing in pinnipeds. J. Acoust. Soc. Am. 1303, 2216-2228.

Kastak, D., Schusterman, R.J., 1999. In-air and underwater hearing sensitivity of a northern elephant seal (*Mirounga angustirostris*). Can. J. Zool. 77, 1751-1758.

Kastak, D., Schusterman, R.J., Southall, D.L., Reichmuth, C.J., 1999. Underwater temporary threshold shift induced by octave-band noise in three species of pinniped. J. Acoust. Soc. Am. 106,1142-1148.

Kastelein, R., P. Mosterd, C.L. van Lightenberg, & W.C. Verboom. 1996. Aerial sensitivity tests with a male Pacific walrus (*Odobenus rosmarus divergens*), in the free field and with headphones. Aquat. Mammals 22, 81-93.

Kastelein, R. Personal communication of preliminary walrus in-water audiometric data.

Ketten, D.R., 1997. Structure and function in whale ears. Bioacoustics 8,103-135.

Ketten, D.R., 2000. Cetacean ears. In: Au, W.W.L., Popper, A.N., Fay, R.R. (Eds.), Hearing by Whales and Dolphins. Springer Handbook of Auditory Research Series. Springer, New York, pp 43-108.

King, J.E., 1983. Seals of the World. 2nd ed. Comstock Publishing Associates, Ithaca.

Kirikae, I., 1960. Density, centre of gravity and axis of rotation of the human auditory ossicles. In: The Structure and Function of the Middle Ear. University of Tokyo Press, Bunkyo-ku, 87-91.

Ledje, C., Arnason, U., 1996a. Phylogenetic analyses of complete cytochrome *b* genes of the order Carnivora with particular emphasis on the Caniformia. J. Mol. Evol. 42, 135-144.

Ledje, C., Arnason, U., 1996b. Phylogenetic relationships within Caniform Carnivores based on analyses of the mitochondrial 12S rRNA gene. J. Mol. Evol. 43, 641-649.

Lees, S., 2001. Elastic properties and measurement techniques of hard tissues (Part I). In: Sinha, D., Levy, M. (Eds.), Handbook of Elastic Properties of Solids, Liquids, and Gases. Volume III: Elastic Properties of Solids: Biological and Organic Materials, Earth and Marine Sciences. Academic Press.

Lees, S., Ahern, J.M., Leonard, M., 1983. Parameters influencing the sonic velocity in compact calcified tissues of various species. J. Acoust. Soc. Am. 74, 28-33.

Lees, S., Hanson, D.B., Page, E.A., 1996. Some acoustical properties of the otic bones of a fin whale. J. Acoust. Soc. Am. 99, 2421-2427.

Lento, G.M., Hickson, R.E., Chambers, G.K., Penny, D., 1995. Use of spectral analysis to test hypotheses on the origin of pinnipeds. Mol. Biol. Evol. 12, 28-52.

Lipatov, N.V., 1992. Underwater hearing in seals: The role of the outer ear. In: Thomas, J., Kastelein, R.A., Supin, A.Y., (Eds.), Marine Mammal Sensory Systems. Plenum Press, New York, pp. 249-256.

Masterton, B., Heffner, H., Ravizza,R., 1969. The evolution of human hearing. J. Acoust. Soci. Am., 54, 966-985.

Mills, R.P., 1993. The use of cortical bone grafts in ossiculoplasty II: Graft mass and hearing change at different frequencies. J. Laryngol. Otol. 107, 690-694.

Møhl, B., 1968a. Auditory sensitivity of the common seal in air and in water. J. Audit. Res. 8, 27-38.

Møhl, B., 1968b. Hearing in seals. In: Harrison, R.J., Schusterman, R.C. (Eds.), The behavior and physiology of pinnipeds, Appleton-Century-Crofts, New York, pp. 172-195.

Møhl, B., Ronald, K., 1975. The peripheral auditory system of the harp seal, *Pagophilus groenlandicus*, (Erxleben, 1777). Rapp. P.-v. Réun. Cons. Int. Explor. Mer 169, 516-523.

Moore, P.W.B., Schusterman, R.J., 1987. Audiometric assesment of northern fur seals, *Callorhinus ursinus*. Mar. Mamm. Sci. 3, 31-53.

Nummela, S., 1995. Scaling of the mammalian middle ear. Hear. Res. 85, 18-30.

Norris, K.S., Leatherwood, K., 1981. Hearing in the bowhead whale, *Balaena mysticetus*, as estimated by cochlear morphology. In Albert, T.F. (Ed.), Tissue structural studies and other investigations on the biology of endangered whales in the Beaufort Sea, 2. U.S. Dept. Interior, Anchorage, pp 745-878.

Oden'hal, S., Poulter, T.C., 1966. Pressure regulation in the middle ear cavity of sea lions: A possible mechanism. Science 153, 768-769.6

Parnell, J.E., Dreher, J.J., 1963. Estimates of auditory frequency response limits as a function of mammalian ossicular density. Lockheed Co. of California Report #17080, pp i-ii, 1-11.

Ramprashad, F., 1975. Aquatic adaptations in the ear of the harp seal P*agophilus groenlandicus* (Erxleben, 1777). Rapp. P.-v. Reun. Cons. Int. Explor. Mer 169, 102-111.

Ramprashad, F., Corey, S., Ronald, K., 1972. Anatomy of the seal's ear (*Pagophilus groenlandicus*) (Erxleben, 1777). In Harrison, R.J. (Ed.), Fuctional Anatomy of Marine Mammals, Volume. 1. Academic Press, London, pp 264-306.

R amprashad, F., Corey, S., Ronald, K., 1973. The harp seal *Pagophilus groenlandicus* (Erxleben, 1777). XIV The gross and microscopic structure of the middle ear. Can. J. Zool. 51, 589-600.

Renouf, D., 1991. Sensory reception and processing in Phocidae and Otaridae. In: Reounf, D. (Ed.), The Behaviour of Pinnipeds. Chapman and Hall: New York, pp. 345-394.

Repenning, C.A., 1972. Underwater hearing in seals: functional morphology. In: Harrison, R.J. (Ed.), Fuctional Anatomy of Marine Mammals, Volume 1. Academic Press, London, pp. 307-331.

Richardson, W.J., 1995. Marine Mammal Hearing. In: Richardson, W.J., Greene, C.R., Malme, C.I., Thomson, D.H. (Eds.) Marine Mammals and Noise. Academic Press, San Diego, pp. 205-240.

Ridgway, S.H., Joyce, P.L., 1975. Studies on seal brain by radiotelemetry. Rapp. P.-v. Réun. Cons. int. Explor. Mer 169, 81-91.

Rosenthal, F., 1825. Uber die sinnesorgane der seehunde. Nova Acta Adademiae Leopoldina 12, 675-695, LVI-LVII.

Schuknecht, H.F., 1993. Pathology of the Ear. Lea and Febiger, Philadelphia. p 31.

Schusterman, R.J., 1974. Auditory sensitivity of a California sea lion to airborne sound. J. Acoust. Soc. Am. 56, 1248-1251.

Schusterman, R.J., Ballet, R.F., Nixon, J., 1972. Underwater audiogram of the California sea lion by the conditioned vocalization technique. J. Exp. Analysis Behav. 17, 339-350.

Schusterman, R.J., Kastak, D., Levenson, D.H., Reichmuth, C.J., Southall, B.L., 2000. Why pinnipeds don't echolocate. J. Acoust. Soc. Am. 107, 2256-2264.

Shaughnessy, P.D. Fay, F.H., 1977. A review of the taxonomy and nomenclature of North Pacific harbor seals. J. Zool. Lond. 182, 385-419.

Southall, B.L., Schusterman, R.J., D. Kastak, D., 2000. Masking in three pinnipeds: Underwater, low-frequency critical ratios. J. Acoutical Soc. Am. 108, 1322-1326.

Tandler, J., 1899. Ueber ein corpus cavernosum tympanicum beim Seehund. Monatsschr. Ohrenheilkd. Kehlkopf-, Nase-, Rachenkr., Organ Oestesr. Otol., Ges., 33 437-440.

Terhune, J.M., 1974. Directional hearing of a harbor seal in air and water. J. Acoust. Soc. Am. 56, 1862-1865.

Terhune, J.M., Ronald, K., 1971. The harp seal, *Papophilus groenlandicus* (Erxleben, 1777). X. The air audiogram. Can. J. Zool. 49, 385-390.

Terhune, J.M., Ronald, K., 1972. The harp seal, *Papophilus groenlandicus* (Erxleben, 1777). III. The underwater audiogram. Can. J. Zool. 50, 565-569.

Terhune, J.M., Ronald, K., 1975. Underwater hearing sensitivity of two ringed seals (*Pusa hispida*). Can. J. Zool. 53, 227-231.

Thomas, J.A., Moore, P., Withrow, R., Stoermer, M., 1990. Underwater audiogram of a Hawaiian monk seal (*Monachus schauinslandi*). J. Acoust. Soc. Am. 87, 417-420.

Thomson, D.H., Richardson, W.J., 1995. Marine Mammal Sounds. In : Richardson, W.J., Greene, C.R., Malme, C.I., Thomson, D.H. (Eds.) Marine Mammals and Noise. Academic Press, San Diego, pp. 159-204.

Tohno, Y., Tohno, S., Utsumi, M., Minami, T., Ichii, M., Okazaki, Y., Nishiwaki, F., Moriwake, Y., Naganuma, T., Yamada, M., Araki, T., 1997. Age-independent constancy of mineral contents in human vertebra and auditory ossicle. Biol. Trace Element. Res. 59, 167-175

Wainwright, W.N., 1958. Comparison of hearing thresholds in air and in water. J. Acoust. Soc. Am., 30, 1025-1029.

Wartzok D., Ketten, D.R., 1999. Marine mammal sensory systems. In J.E. Reynolds III, J.E., Rommel, S.A. (Eds.), Biology of marine mammals. Smithsonian Institution Press, Washington, pp 117-175.

White, M.J., Jr., Norris, J., Ljungblad, D., Baron, K., and di Scara, G. 1978. Auditory threshold of two beluga whales,(*Delphinapterus leucas*). Hubbs/Sea World Res. Inst. Tech. Rep. 78-109 for Naval Ocean Systems Center, San Diego, CA.

Wyss, A.R., 1987. The walrus auditory region and the monophyly of pinnipeds. Am. Mus. Novitates, 2871, 1-31.

Yokoyama, T., Iino, Y., Kakizaki, K., Murakami, Y., 1999. Human temporal bone study on the postnatal ossification process of auditory ossicles. Laryngoscope 109, 927-930.

Yost, W.A., 1994. Fundamentals of hearing: An introduction. Academic Press: San Diego.

Zuckerkandl, E., 1896. Beiträge zur vergleichenden Anatomie der Ohrtrompete: II. Monatchr. F. Ohrenheilk., 30, 105-112.